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Antibiotic Toxicity, Efficacy, and Teratogenicity in Adult Spring Chinook Salmon (*Oncorhynchus tshawytscha*)

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DECEW, M. G. 1972. Antibiotic toxicity, efficacy, and teratogenicity in adult spring chinook salmon (*Oncorhynchus tshawytscha*). J. Fish. Res. Bd. Canada 29: 1513–1517.

Antibiotics were tested for toxicity and efficacy in adult spring chinook salmon (*Oncorhynchus tshawytscha*) infected with bacterial kidney disease and furunculosis. The complex of penicillin G procaine, dihydrostreptomycin sulfate, and oxytetracycline–HCl was not toxic and it effectively controlled both diseases, producing a three-fold increase in adult survival and production of viable eggs.

Mandible and fin teratogenesis occurred in progeny of adults treated with the above antibiotic complex, but could be reduced by providing a 32 day interim between injection and spawning.

DECEW, M. G. 1972. Antibiotic toxicity, efficacy, and teratogenicity in adult spring chinook salmon (*Oncorhynchus tshawytscha*). J. Fish. Res. Bd. Canada 29: 1513–1517.

L'auteur vérifie la toxicité et l'efficacité d'antibiotiques sur des saumons chinook de printemps adultes (*Oncorhynchus tshawytscha*) atteints d'une maladie bactérienne du rein et de la furonculose. Le complexe pénicilline G procaïne, dihydrostreptomycine sulfate et oxytétracycline-HCl n'est pas toxique, contrôle efficacement les deux maladies et triple les chances de survie des adultes et de production d'oeufs viables.

La progéniture des adultes traités au complexe antibiotique décrit plus haut exhibe une tératogénèse de la mandibule et des nageoires, qui peut être atténuée par un délai de 32 jours entre l'inoculation et la fraie.

Received April 18, 1972

ADULT spring chinook salmon (*Oncorhynchus tshawytscha*) migrate in April through June to the Cowlitz River Salmon Hatchery in Washington. During a 5-month holding period, until spawning, they become infected with bacterial kidney disease (*Corynebacterium sp.*), columnaris disease (*Chrondrococcus columnaris*), and furunculosis disease (*Aeromonas salmonicida*), resulting in 74% mortality.

The feasibility of controlling systemic infections in adult salmon was indicated by previous studies. Antibiotics were used to control infectious dropsy in adult carp (Schäperclaus 1956). Administration

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of sulfadimethoxine intramuscularly to adult salmon produced therapeutic blood levels, but caused sterile abscesses at the injection site. When given intraperitoneally to adult female salmon it caused excessive loss of the progeny (Amend and Fryer 1968).

Experiments were initiated in 1968 to test a group of antibiotics for toxicity and then selected antibiotics were tested for efficacy in an attempt to reduce adult prespawning losses. Due to the occurrence of deformed progeny, further tests were conducted on efficacy and teratogenicity.

Materials and Methods

Toxicity

Antibiotics were administered to eight lots (five males and five females per lot) of adult spring chinook salmon. The antibiotics and dosages per kg of body weight were: Lot 1, penicillin G procaine (22,000 IU); Lot 2, oxytetracycline–HCl (5 mg); Lot 3, penicillin G procaine (22,000 IU) and dihydrostreptomycin sulfate (28 mg); Lot 4, penicillin G procaine (17,000 IU), benzathine penicillin G (17,000 IU), and dihydrostreptomycin sulfate (28 mg); Lot 5, penicillin G procaine (22,000 IU), dihydrostreptomycin sulfate (28 mg), and oxytetracycline–HCl (5 mg); Lot 6, erythromycin (5 mg); Lot 7, neomycin sulfate (11 mg); and Lot 8, saline (1 mg). The adults had an average weight of 9 kg and the drugs were injected subcutaneously between the dorsal ridge and musculature approximately 12 mm anterior to the dorsal fin. Water temperature was 12–15 C and the test lasted 10 days. Mortalities were examined microbiologically and tissues from four surviving adults of each lot were examined grossly for toxicity signs. Microbiological examinations consisted of inoculating kidney tissue on trypticase soy agar and Ordal's bacterial kidney disease medium (Bullock 1961). These media were incubated 3 and 10 days, respectively, at 20 C. Kidney smears were stained by the Gram's method.

Efficacy and Teratism

A mixture of penicillin G procaine, dihydrostreptomycin sulfate, and oxytetracycline–HCl (PSO)[1] was tested for efficacy against bacterial kidney disease and furunculosis in adult spring chinook during three experiments (1968–70). In each experiment the infected population was randomly divided into three lots, providing an equal number of adults per lot. The number of females per lot was subsequently determined following development of secondary sex characteristics. As many as 1500 adults (weighing 2–16 kg) per day were injected subcutaneously, as described above, using two automatic syringes. PSO was compounded by mixing equal parts of Combiotic and Liquamycin (Pfizer), both containing additives as well as the antibiotic components. In each experiment, two lots received PSO and one served as the untreated control. During injection the adults were anesthetized with tricaine methane sulfonate (57 ppm) in 1968 and 1969 and ethyl-*p*-aminobenzoate (40 ppm) in 1970. Malachite green (0.75 ppm) was administered to the adults for control of *C. columnaris* and external fungus. The incubating eggs were also treated with malachite green (2 ppm) for fungus control. Spawning occurred from approximately September 6 to October 10. Adult mortalities were examined microbiologically as described above. Egg productivity was evaluated on an equivalent basis by computing the average number of viable eggs per adult female initially distributed to each lot. Juvenile progeny were grossly examined for deformities, using sample sizes determined in accordance with the binomial sampling theorem (Dixon and Massey 1957).

Experiment 1968 — Lot 2 was injected on September 4 and 16 and Lot 3 on August 20 and September 6 and 17. Non-sexually mature adults were injected concurrent with the spawning of other adults and these injected individuals were spawned 7–14 days later, providing a minimum time for drug elimination.

Experiment 1969 — Lot 2 was injected on August 7 and 22. The injection dates for Lot 3 were July 24 and August 13 and 28. These schedules provide a greater interim between injection and spawning periods than occurred in the previous experiment, with Lot 2 having the longer interim.

Experiment 1970 — Three injections were administered to each lot at 21 day intervals. The injection dates for Lot 2 were June 24, July 16, and August 7; and for Lot 3 were July 15 and August 6 and 27. Lot 2 had the longer interim between the last injection and the spawning period.

Table 1 shows details of the experimental design.

Results

Toxicity

None of the seven antibiotic formulations produced evidence of acute toxicity. All 80 adults survived, except one male in the neomycin treated lot and one female in the control lot. The dermis surrounding the injection site turned dark soon after injection, regaining its normal pigmentation within 3 days. No indication of toxicity was seen in the gross examination of visera and muscle tissue from four surviving adults of each lot. Subcutaneous injection of the seven antibiotic formulas produced minimal hemorrhaging at the injection site and no sterile abscesses.

Efficacy

PSO controlled the mixed infection of furunculosis and bacterial kidney disease in each of the three

[1]PSO is an abbreviation for the three antibiotics.

experiments[2] (Table 2). The sequence of three injections increased adult survival by 43, 24, and 160%; and viable egg production by 50, 30, and 250% in 1968, 1969, and 1970, respectively. Two injections increased adult survival and egg production (in 1969) to a lesser extent. Post-spawning egg loss was lower in the treated lots. Necrotic ovaries, apparently caused by the infectious process, were frequently observed during spawning of the control lots; while this condition was rarely seen in the treated lots.

Microbiological examination showed that PSO therapy resulted in less bacterial kidney disease and furunculosis among the mortalities, indicating control of the diseases. In 1968 and 1970, mortalities from the untreated control had a high incidence of these diseases throughout the holding period; while examination of mortalities from the treated lots indicated that following therapy bacterial kidney disease had been reduced and furunculosis eliminated (Table 3). The incidence of both diseases prior to

[2]The experimental lots produced 1.7, 4.0, and 2.3 million eggs in 1968, 1969, and 1970, respectively.

TABLE 1. Experimental design of tests conducted on the efficacy and teratogenicity of PSO administered to adult spring chinook salmon.

	Experimental year		
	1968	1969	1970
No. adults/lot	410	640	695
Water temp. range (*c*)	12–15	8–13	9–12
Vol. PSO/injection (*ml*)	2.0	2.0	0.7, 1.4, 2.0[a]
Dosage/kg body wt.			
penicillin G procaine (*IU*)	13,000–87,000	13,000–87,000	15,000–30,000
dihydrostreptomycin sulfate (*mg*)	16–109	16–109	19–38
oxytetracycline–HCl (*mg*)	3–22	3–22	4–8
Days between last injection and initial spawning			
Lot 1	Control	Control	Control
Lot 2	0	14	32
Lot 3	0	8	12

[a]In 1970, adults within progressive body weight intervals received corresponding proportionate volumes of PSO.

TABLE 2. Antibiotic therapy, adult survival, egg mortality, viable eggs per original female, and deformed fry. Adult spring chinook injected with PSO in 1968–70.

Year	Lot	No. of injections	Interim (*days*)[a]	% adult survival	% post-spawning egg loss	No. viable eggs/original female	% deformed fry[b]
1968	1	0	–	56	12.3	1833	0
	2	2	0	66		Data not collected	
	3	3	0	80	8.1	2720	15
1969	1	0	–	66	10.8	2015	0
	2	2	14	74	4.5	2435	2
	3	3	8	82	3.8	2640	9
1970	1	0	–	33	23.0	781	0
	2	3	32	89	3.5	2760	0.1
	3	3	12	86	3.6	2730	3.6

[a]The number of days between injection and spawning periods.
[b]A total of 1500, 13,500, and 20,000 juveniles were examined in 1968–70, respectively.

therapy was also determined in 1969. Kidney tissue smears from adult mortalities that had been treated with PSO contained few bacterial kidney disease organisms and these were often broken or swollen; while kidney tissue smears from the control mortalities contained large masses of morphologically normal organisms.

Teratism

PSO treatment of the adults, especially close to spawning, caused developmental anomalies in the progeny. The mandible of anomalous yearlings (120 mm total length) was 1–6 mm shorter than normal. Frequently the caudal fin was narrower and the anal fin shorter than normal. The incidence of these deformities was excessive in 1968, but it was reduced to a minimum by 1970 (Table 2).

The incidence of teratogenic progeny was related to the time lapse between the last injection and the first spawning date. In 1969, the incidence was greater in progeny produced in the first 15 days of the spawning period than in the last 15 days, regardless of whether two or three injections had been administered to the adults (Table 4). This table also shows that the incidence of deformities was relatively constant between the fry, fingerlings, and yearling stages; suggesting that most of the anomalous juveniles experience a normal survival rate. The deformed fish also appeared to grow at a normal rate. Data from the 1970 study (Table 2) showed that administering three injections with a 32 day interim between injection and spawning periods resulted in fewer deformities (0.1%) than the same number of injections followed by a 12 day interim (3.6% deformities). The 3.6% incidence occurred even though the dosage was lower in 1970 than in 1969, suggesting dosage is not the dominant factor. In 1970, most of the deformities were produced by the early part of the spawning period. Lot 2 had 1% incidence for the first spawning date and 0% thereafter and Lot 3 had 18, 2, 0, 2, and 0% incidence through the five spawning dates (1500–3000 fry sampled for each date and lot).

Table 3. Percent of adult mortalities infected with bacterial kidney disease (BKD) and furunculosis (Fur).[a]

Experiment	1968		1969		1970	
Diseases	BKD	Fur	BKD	Fur	BKD	Fur
Before therapy	100	55	70	15	100	20
After therapy						
Control	100	50	Data not		100	17
Treated	45	0	collected		30	0

[a]A total of 60, 20, and 90 adults were examined in 1968, 1969, and 1970, respectively.

Discussion

The efficacy of PSO against bacterial kidney disease and furunculosis allowed survival and successful spawning of the adults, proving progeny necessary for the continuance of the race of salmon. The study also demonstrated in vivo sensitivity of the bacterial kidney disease organism to the PSO mixture of antibiotics. The occurrence of swollen, broken, and reduced numbers of bacterial kidney disease organisms in tissue from fish treated with PSO is consistent with the action of penicillin against gram positive organisms as described by Burrows (1964).

The incidence of mandible and fin anomalies was reduced to a minimum, providing a measure of confidence in the use of PSO. However, it is conceivable that it could cause currently undetected (physiologic) anomalies in the progeny. The total effect that PSO has on the progeny could be eval-

Table 4. Deformity incidence produced by first and second halves of the spawning period in progeny of 1969 adult spring chinook treated with PSO.[a]

Lot	No. of Injections	Period: Sept. 9–24 % deformed			Period: Sept. 25–Oct. 8 % deformed		
		Fry	Fingerlings	Yearlings	Fry	Fingerlings	Yearlings
1	0	0	0	0	0	0	0
2	2	3	4	–[b]	1	1	1
3	3	18	15	13	1	4	4

[a]Each of the 18 samples contained 750 juveniles.
[b]Data not collected.

uated by marking these juveniles and enumerating survival to adulthood, upon capture in the ocean, river, or hatchery facility. The 32 day interim between injection and spawning periods contraindicates PSO therapy in salmon species that are near sexual maturity when they migrate to the hatchery.

In addition to PSO therapy, the adults received tricaine methane sulfonate (1968 and 1969), ethyl-*p*-aminobenzoate (1970), and malachite green (1968–70). Because they were administered equally to the control and treated lots, and mandible and fin anomalies occurred only in the latter, these chemicals are not suspected to be teratogens.

The occurrence of PSO teratogenicity in salmon suggests the potential of using fish in a teratologic test system for screening human chemotherapeutics. Current teratologic studies generally employ avian or mammalian systems that are physiologically more closely related to human beings, but are expensive, time consuming, and involve relatively few progeny. Similar studies with salmon are more readily performed and provide a large number of progeny per parent, lending greater statistical significance. The salmon teratologic test could be used to augment avian and mammalian studies.

Current recommendations for administering PSO to salmon are: (1) the administration of PSO should be contingent upon sufficient need for therapy in relation to the risk of teratogenesis; (2) the sequence of three PSO injections should be scheduled with a 32 day interim between the last injection and first day of spawning; (3) the recommended dosage is 15,000–30,000 IU penicillin G procaine, 19–38 mg dihydrostreptomycin sulfate, and 4–8 mg oxytetracycline–HCl per kg of body weight; (4) carcasses of PSO treated adults should not be used as human or animal food; and (5) the total effect of PSO therapy on the progeny of the treated adults should be evaluated by adult mark–return studies.

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AMEND, D. F., AND J. L. FRYER. 1968. The administration of sulfonamide drugs to adult salmon. Progr. Fish-Cult. 30: 168–172.

BULLOCK, G. L. 1961. A schematic outline of the presumptive identification of bacterial diseases of fish. Progr. Fish-Cult. 23: 147–151.

BURROWS, W. 1964. Textbook of microbiology. W. B. Saunders Co., Philadelphia, Pa. 1155 p.

DIXON, W. J., AND F. T. MASSEY. 1957. Introduction to statistical analysis. McGraw Hill, New York, N.Y. 488 p.

SCHÄPERCLAUS, W. 1956. Striking results obtained in the treatment and control of infectious dropsy in carp with antibiotics. Sport Fish. Abstr. 2 (1): 8.

Methylmercury in Northern Pike (*Esox lucius*): Distribution, Elimination, and Some Biochemical Characteristics of Contaminated Fish

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Northern pike heavily contaminated with methylmercury were captured from Clay Lake, Ontario, and released in Heming Lake, Manitoba, an area relatively free of mercury. Mercury levels in muscle biopsy samples at the time of transfer and at subsequent recaptures indicated that only 30% was eliminated in one year. Distribution among various body tissues was essentially unchanged, those organs most heavily contaminated being lens, kidney, and liver in decreasing order. Biochemical profiles of blood serum constituents showed several differences between samples from the two lakes, especially in levels of inorganic phosphate, total protein, alkaline phosphatase, and cortisol. Serum values for transplanted fish tended toward those in the clean lake and we have concluded that biochemical profiles were sensitive to the environmental change.

LOCKHART, W. L., J. F. UTHE, A. R. KENNEY, AND P. M. MEHRLE. 1972. Methylmercury in northern pike (*Esox lucius*): distribution, elimination, and some biochemical characteristics of contaminated fish. J. Fish. Res. Bd. Canada 29: 1519–1523.

Des grands brochets fortement contaminés par le mercure-méthyl ont été capturés dans le lac Clay, Ontario, et relâchés dans le lac Heming, Manitoba, une région relativement libre de Hg. Les concentrations de Hg dans des échantillons de biopsie du muscle au moment du transfert et lors de recaptures subséquentes indiquent que seulement 30% est éliminé en une année. La répartition du Hg dans les divers tissus demeure essentiellement la même, le cristallin, le rein, et le foie étant, par ordre décroissant, les organes les plus lourdement contaminés. Les profils biochimiques des paramètres du sérum sanguin d'échantillons provenant des deux lacs montrent plusieurs différences, particulièrement sous le rapport phosphate inorganique, protéine totale, phosphatase alcaline, et cortisol. Les valeurs observées dans le sérum des poissons transplantés se rapprochent de celles du lac non contaminé. Nous en concluons que les profils biochimiques sont sensibles aux variations de l'habitat.

Received April 25, 1972

MERCURY contamination in Canadian fish was reported by Wobeser et al. (1970) and has been surveyed by Bligh (MS 1970). Laboratory studies (Jarvenpaa et al. 1970) have indicated that existing mercury in northern pike (*Esox lucius*) is cleared very slowly (half-life 640–780 days). We wished to determine whether the half-life was equally long in nature and therefore we transplanted fish from a contaminated lake to a relatively uncontaminated one to measure the rate of mercury loss. Westoo (1966) first reported that mercury in pike occurred predominantly as methylmercury and we have analyzed body tissues for both total mercury and methylmercury.

We wished also to determine whether the biochemistry of contaminated fish had been significantly affected. We selected the approach used by Eisler (1967) with puffers and obtained biochemical profiles

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of several blood serum constituents on transplants and on samples native to both lakes.

Materials and Methods

In November, 1970, 400 northern pike were taken by gillnetting from Clay Lake, Ontario (50°03′N, 93°30′W), and transplanted to Heming Lake, Manitoba (54°53′N, 101°07′W). Transportation was by truck and train in an 1100-liter tank aerated with compressed air and there was little mortality (12/400). Before release fish were anesthetized with M.S. 222, weighed, measured, tagged, and biopsied by the method of Uthe (1971).

Fish were recaptured periodically by gillnetting throughout 1971, and in October live samples of transplanted fish were taken together with samples of native populations from both lakes. Fish were taken from the gillnets (3-inch mesh) and transferred to a submerged cage where they were held until sampled. Fish from Clay Lake were found to be 3–8 years of age while those from Heming Lake were 2–7 years old. Blood samples were obtained from caudal vessels by inserting a 20-gauge needle adjacent to the anal fin (Steucke and Schoettger 1967). Blood was allowed to clot at ambient temperature for up to 6 hr; serum was withdrawn and stored at 0 C until analyzed (within 48 hr) by a commercial laboratory using Technicon SMA 12/60 methodology. The reproducibilities for eight replicate samples and the recoveries of internal standards are shown below:

	Reproducibility (%)	Recovery (%)
Inorganic phosphate	0.5	73
Glucose	1.3	97
BUN	2.9	92
Uric acid	0.7	112
Total protein	8.4	112
Alkaline Phosphatase	0.7	103

Serum cortisol was measured according to the fluorometric method of Mattingly (1962) on 50 μl of serum. The reproducibility of eight replicate samples was 1.3%, and the recovery was 98%. Additions of 11-deoxycortisol, corticosterone, progesterone, and cortisone before extraction failed to increase the final fluorescence readings.

Muscle and bile samples were taken before the remainder of the fish was frozen and returned to the laboratory for dissection and analyses of other tissues. Total mercury concentrations were determined by the atomic absorption method of Armstrong and Uthe (1971). Methylmercury was measured by the chromatographic procedure of Uthe et al. (1972). Mercury content per fish was estimated as the product of muscle concentration and fish weight. The calculated amount of mercury was compared with that actually found by homogenizing six whole fish and the estimates ranged from 97.9% to 106.3% of that found, as indicated below:

Mercury estimated as the product of biopsy concentration and weight (μg)	Mercury found by sampling homogenized whole fish (μg)
3690	3770
9060	8990
4040	3800
5240	5210
7180	7250
11,400	11,300

Results and Discussion

Total mercury levels in fish recaptured at intervals throughout 1971 are shown in Table 1. These results suggest a half-life of about 2 years for mercury in pike which agrees with the laboratory studies of Jarvenpaa et al. (1970).

One female was recaptured on three occasions and the mercury concentrations found are shown below:

	Wt (g)	Biopsy level (μg/g)	Total mercury (μg)
Nov., 1970	1000	8.43	8430
July 21, 1971	1970	4.34	8560
July 31, 1971	2160	3.83	8380
Oct., 1971	2590	2.31	5980

TABLE 1. Mercury content of northern pike (*Esox lucius*) at intervals after transplanting them from Clay Lake, Ont., to Heming Lake, Man.

Sample date	No.	Original weight (g)	Original mercury (μg)	Recapture weight (g)	Recapture mercury (μg)
Jan., 1971	9	9560	67,200	9370	70,600
(3 months)					(105%)
April, 1971	18	16,160	140,800	16,210	117,200
(6 months)					(83%)
July, 1971	17	18,500	146,000	22,040	120,300
(9 months)					(82%)
Oct., 1971	5	3920	32,700	8360	23,100
(12 months)					(71%)

At the time of the final biopsy the mercury concentration in white muscle was reduced to 27% of its original value, while total Hg remaining in the animal exceeded 70%. It seems that dilution by growth was as important as elimination in reducing muscle concentration.

Indications of loss and dilution were not artifacts of redistribution among body tissues. Several tissues were analyzed from Clay Lake and transplanted fish, and results were expressed as ratios of mercury in a given tissue to that in white muscle from the same fish (Table 2). Kidney tissue gave poor reproducibility since it tended to degenerate on freezing and thawing. White muscle levels of mercury from Clay Lake fish ranged from 6.29 to 16.0 μg/g (wet weight), while that in transplants ranged from 6.82 to 13.75 μg/g at the time of transfer, and from 1.69 to 11.4 μg/g at recapture. Samples from native Heming Lake pike were all below 0.3 μg/g. In general, the tissue ratios were similar and indicated few differences in distribution between fish from Clay Lake and those transplanted to Heming Lake. There are several differences between these results and those reported by Johnels et al. (1967) for pike from Sweden, but levels of contamination were considerably lower there. The greatest changes during the year were in lens and scales in which ratios to white muscle doubled; however, since these tissues were not biopsied at the time of transplant, we cannot distinguish whether mercury shifted to them or whether they simply retained original levels and grew more slowly than muscle.

TABLE 2. Distribution of total mercury in tissues of northern pike (*Esox lucius*) as a ratio of tissue concentration/white muscle concentration. Values are means ± SD; number of samples in parentheses.

Tissue	Clay Lake	Transplanted from Clay to Heming
Red muscle	1.01±0.08(5)	0.99±0.16(12)
Liver	1.57±0.42(5)	1.50±0.37(12)
Kidney	2.16±0.77(5)	1.64±0.68(12)
Spleen	1.11±0.08(5)	0.86±0.32(10)
Stomach	0.75±0.17(5)	0.92±0.23(12)
Intestine	1.49±0.21(5)	1.32±0.26(11)
Brain	0.74±0.10(5)	0.66±0.15(12)
Gills	0.52±0.09(5)	0.46±0.10(12)
Skin	0.30±0.03(5)	0.35±0.06(7)
Ovary	0.39±0.08(4)	0.37±0.19(3)
Testis	0.59±0.07(2)	0.62±0.03(4)
Heart	1.22±0.17(5)	1.04±0.45(8)
Scales	0.26±0.03(5)	0.54±0.28(7)
Lens	2.42±1.66(5)	5.77±2.93(3)
Blood	0.22±0.05(4)	0.27±0.01(3)
Bile	0.12±0.08(3)	0.17±0.12(3)

The proportion of methylmercury in various tissues is shown in Table 3. Values from transplanted fish were often slightly lower, but there were no large changes; methylmercury remained the predominant form in all tissues studied, as was reported by Westoo (1966). Methylmercury in bile is of interest since it represents a possible excretory mechanism. Takahashi and Hirayama (1971) were able to prevent intestinal reabsorption of mercury in mice by feeding indigestible thiol containing compounds which accelerated methylmercury elimination. It seems likely that intestinal reabsorption of bile methylmercury occurs in pike and contributes to its long half-life.

Pike from Clay Lake showed no macroscopic evidence of damage with the exception of some emaciation. Histological examination of liver, kidney, spleen, and gill tissues revealed no structural damage, although fat storage in liver was much reduced as compared with Heming Lake and transplanted pike. (B. Hobden personal communication).

In the absence of obvious physical signs of methylmercury poisoning, biochemical profiles were determined using the methods of human clinical chemistry. Hunt (1970) reported that Technicon Autoanalyzer methodology was successful with fish blood and Christensen et al. (1972) have used an

TABLE 3. Fraction of total mercury found to be present as methylmercury (%) in several tissues of northern pike (*Esox lucius*) from Clay Lake, Ont., and 1 year after transplant to Heming Lake, Man. (Percentages are means of determinations from two to five individuals except as noted.)

Tissue	Clay Lake	Transplanted from Clay to Heming
White Muscle	105	100
Red Muscle	98	98
Liver	97	82
Posterior Kidney	104	94
Spleen	91	92
Stomach	102	80
Intestine	97	85
Brain	87	63
Gills	102	89
Skin	111	85
Ovary	99	92
Testis	93	102[a]
Heart	63	82
Scales	61	83
Lens	112	94
Blood	89	89[a]
Bile	102	103

[a]One sample.

TABLE 4. Serum values for northern pike (*Esox lucius*) from Clay Lake, Ont., and Heming Lake, Man. Transplants were analyzed 1 year after being moved from Clay Lake to Heming Lake.

	Inorganic Phosphate (*mg* % P)	Glucose (*mg* %)	BUN (*mg* %)	Uric acid (*mg* %)	Total Protein (*g* %)	Alkaline phosphatase (*mμ/ml*)	Cortisol (*μg* %)
Clay Lake							
mean	11.0	115	3.4	2.2	3.9	51	25.0
SD	2.4	74	1.8	1.1	0.7	23	15.6
range	7.2–17.2	20–360	2.0–7.0	0.4–5.2	2.4–5.4	14–130	8–64
Heming Lake							
mean	8.4	280	2.4	2.6	4.8	92	96.3
SD	1.2	152	1.4	1.2	0.9	19	41.2
range	5.5–9.8	25–550	1.0–5.0	1.4–7.0	3.5–7.7	48–140	56–148
Transplants							
mean	6.6	435	2.3	1.7	4.5	64	54.0
SD	1.5	167	0.5	0.3	0.7	8.0	19.4
range	5.4–8.8	195–550	2.0–3.0	1.5–2.2	3.8–5.5	57–74	32–74
t' (for difference between Clay and Heming means)	4.95[b]	2.45[a]	2.47[a]	0.91	3.58[b]	5.95[b]	4.13[b]

[a] $P < .05$.
[b] $P < .01$.

Autoanalyzer in their studies of copper toxicity in the brown bullhead. Serum was analyzed for several constituents and the results are given in Table 4. Possible causes for the observed differences are speculative, but decreases in levels of glucose, total protein, and alkaline phosphatase have been described in cases of human starvation (Davidsohn and Henry 1969); and low Clay Lake values for these constituents may have related to the emaciation observed there. Grant and Mehrle (1970) observed that endrin interfered with feeding behavior in goldfish and that exposed fish had decreased levels of serum protein.

Serum cortisol values were significantly lower ($P < 0.01$) in fish from Clay Lake than in either transplanted or native Heming Lake fish. The lower levels of cortisol suggest that either interrenal secretion of cortisol was suppressed, or that the rate of clearance of cortisol was altered. B. F. Grant and P. M. Mehrle (unpublished data) observed a similar decrease in rainbow trout chronically exposed to dietary endrin. Cortisol levels in the transplanted fish were significantly lower than those in the Heming fish, but significantly greater than those in Clay Lake. This suggests that after 1 year the transplanted fish were recovering, but were still significantly affected. Alteration of this stress–response mechanism implies lowered adaptability and survival by the reduced ability to respond to environmental stresses such as disease, predation, and physical extremes in water quality. Mercury cannot be cited as a direct cause of any biochemical differences observed here since the two lakes differ in other ecological factors. Lawler and Watson (1958) have described Heming Lake, and preliminary information on Clay Lake is available (German MS 1969).

These studies indicate that methylmercury is lost only slowly from northern pike and that there is little redistribution among body tissues. The biochemical measurements indicate internal differences between Clay Lake and Heming Lake pike, and values for transplanted fish suggest that biochemical differences can be induced by environmental changes.

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ARMSTRONG, F. A. J., AND J. F. UTHE. 1971. Semi-automated determination of mercury in animal tissue. At. Absorpt. Newsl. 10: 101–103.

BLIGH, E. G. MS 1970. Mercury and the contamination of freshwater fish. Fish. Res. Board Can. MS Rep. 1088: 27 p.

CHRISTENSEN, G. M., J. M. MCKIM, W. A. BRUNGS, AND E. P. HUNT. 1972. Changes in the blood of the brown bullhead (*Ictalurus nebulosus* (Lesueur)) following short and long-term exposure to copper (II). Toxicol. Appl. Pharmacol. (In press)

DAVIDSOHN, I., AND J. B. HENRY. 1969. Clinical diagnosis by laboratory methods. W. B. Saunders Co., Philadelphia, Pa.

EISLER, R. 1967. Tissue changes in puffers exposed to methoxychlor and methyl parathion. U.S. Bur. Sport Fish. Wildl. Tech. Pap. 17: 15 p.

GERMAN, M. J. MS 1969. A water pollution survey of the Wabigoon River. Ont. Water Resour. Comm. MS Rep. 24 p.

GRANT, B. F., AND P. M. MEHRLE. 1970. Chronic endrin poisoning in goldfish, *Carassius auratus*. J. Fish. Res. Bd. Canada 27: 2225–2232.

HUNT, E. P. 1970. Use of a 3 channel autoanalyzer I to measure enzymes and other chemical constituents in the blood of fish. Presented at Technicon Int. Congr., Tarrytown, N.Y.

JARVENPAA, T., M. TILLANDER, AND J. K. MIETTINEN. 1970. Methylmercury: half-time of elimination in flounder pike and eel. Suom. Kem. B 43: 439–442.

JOHNELS, A. G., T. WESTERMARK, W. BERG, P. I. PERSSON, AND B. SJOSTRAND. 1967. Pike (*Esox lucius* L.) and some other aquatic organisms in Sweden as indicators of mercury contamination in the environment. Oikos 18: 323–333.

LAWLER, G. H., AND N. H. F. WATSON. 1958. Limnological studies of Heming Lake, Manitoba and two adjacent lakes. J. Fish. Res. Bd. Canada 15: 203–218.

MATTINGLY, D. 1962. A simple fluorometric method for the estimation of free 11-hydroxy corticoids in human plasma. J. Clin. Pathol. (London) 15: 374–379.

STEUCKE, E. W., JR., AND R. A. SCHOETTGER. 1967. Comparison of three methods of sampling trout blood for measurement of hematocrit. Progr. Fish-Cult. 67: 98–101.

TAKAHASHI, H., AND K. HIRAYAMA. 1971. Accelerated elimination of methylmercury from animals. Nature 232: 201–202.

UTHE, J. F. 1971. A simple field technique for obtaining small samples of muscle from living fish. J. Fish. Res. Bd. Canada 28: 1203–1204.

UTHE, J. F., J. SOLOMON, AND B. GRIFT. 1972. A rapid semimicro method for the determination of methylmercury in fish tissue. J. Ass. Offic. Anal. Chem. 55: 583–589.

WESTOO, G. 1966. Determination of methylmercury compounds in foodstuffs. I. Methylmercury compounds in fish, identification and determination. Acta Chem. Scand. 20: 2131–2136.

WOBESER, G., N. O. NIELSEN, R. H. DUNLOP, AND F. M. ATTON. 1970. Mercury concentrations in tissues of fish from the Saskatchewan River. J. Fish. Res. Bd. Canada 27: 830–834.

Respiratory Adaptations of Prenatal Young in the Ovary of two Species of Viviparous Seaperch, *Rhacochilus vacca* and *Embiotoca lateralis*

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Webb, P. W., and J. R. Brett. 1972. Respiratory adaptations of prenatal young in the ovary of two species of viviparous seaperch, *Rhacochilus vacca* and *Embiotoca lateralis*. J. Fish. Res. Bd. Canada 29: 1525–1542.

The structure of the ovary and embryos of embiotocids is reviewed in relation to those factors that would affect the exchange of materials between ovarian and embryonic blood streams. Weight, exchange surface areas (particularly of the fins), diffusion distances, and capillary densities were measured for prenatal young and ovaries of two species of Embiotocidae, *Rhacochilus vacca* and *Embiotoca lateralis*, during middle and late gestation. Brood size and ovarian fluid volume were also measured. Changes in brood weight and ovarian fluid volumes were interpreted in relation to the load imposed on the ovary by the young. This was expressed as the brood/ovary weight ratio which was found to increase during gestation to a maximum value of eight. Changes in exchange surface areas and diffusion distances of the young and the ovary were determined and evaluated in relation to the metabolic requirements of the brood. Convection of the ovarian fluid must occur as a necessary mechanism for efficient exchange of materials with increasing brood/ovary weight ratio. It is further concluded that the supply of materials to the brood would be limited by ovarian blood-flow characteristics rather than structural considerations, except immediately prior to parturition.

Webb, P. W., and J. R. Brett. 1972. Respiratory adaptations of prenatal young in the ovary of two species of viviparous seaperch, *Rhacochilus vacca* and *Embiotoca lateralis*. J. Fish. Res. Bd. Canada 29: 1525–1542.

Les auteurs passent en revue la structure de l'ovaire et des embryons d'Embiotocidae en fonction des facteurs qui affectent l'échange de matériaux, par voie du flot sanguin, entre l'ovaire et l'embryon. Ils déterminent le poids, la superficie des surfaces d'échange (surtout des nageoires), la distance de diffusion et la densité capillaire des embryons et des ovaires chez deux espèces d'Embiotocidae, *Rhacochilus vacca* et *Embiotoca lateralis*, au milieu et à la fin de la gestation. Ils déterminent de plus le poids de la portée et le volume du liquide ovarien. Les variations de ces deux paramètres sont interprétées en fonction de la charge imposée sur l'ovaire par les jeunes, et exprimées sous forme de quotient poids de la portée/poids de l'ovaire. Au cours de la gestation, ce rapport atteint une valeur maximale de huit. Les variations de superficie des surfaces d'échange et des distances de diffusion des jeunes et de l'ovaire sont évaluées par rapport aux exigences métaboliques de la portée. La convection du liquide ovarien est un mécanisme nécessaire à l'échange efficace des matériaux à mesure que le quotient pondéral portée/ovaire augmente. Les auteurs concluent de plus que l'approvisionnement des embryons en matériaux est limité par les caractéristiques du flot sanguin de l'ovaire plutôt que par des considérations structurales, sauf immédiatement avant la parturition.

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Viviparity in fish is always associated with a decrease in the number of eggs coupled with an increase in protection and nutrition of the young. Numerous adaptations have evolved for the care and maintenance of the young during intraovarian life (Amoroso 1960). Viviparity undoubtedly evolved from an oviparous condition, as in many unspecialized Poeciliidae (Turner 1940a) where the usual nutritional requirements prior to a free-living existence are present in the egg. In less specialized, intermediate ovoviviparous Poeciliidae, the same nutritional situation is found, except that the parent meets the respiratory and excretory needs of the young by using those embryonic exchange structures that are found in their oviparous counterparts. In other families of teleosts, including the specialized ovoviviparous Poeciliidae and Anablepidae, and the viviparous Jenynsidae, Goodeidae, Brotulidae,

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and Embiotocidae, there is a trend in each family towards greater dependence on the parent for all the needs of the young (Turner 1933, 1938a, b, 1940a, b, c, d). It is among these groups that the greatest adaptive mechanisms have evolved to meet the demands of the young and to increase the efficiency of exchange between the parent and the brood.

The comparative anatomy of embryonic and ovarian structures of viviparous fish has been described in detail (reviewed by Amoroso 1960, and Hoar 1969). It is apparent that two critical stages in development require special physiological adaptations of the ovary and the brood. The first stage occurs when the yolk is fully absorbed and further nutritional requirements have to be met. The second occurs when the respiratory requirements exceed the capacity of the unspecialized exchange surfaces. In many cases increase in embryonic surfaces occurs but in some, notably in the Jenynsidae, only ovarian adaptions are involved.

In those young where secondary embryonic surfaces are evolved that persist until after birth, as in embiotocids and some goodeids, a third critical stage can be recognized at birth. Elaborated embryonic surfaces must become reduced or nonfunctional to avoid excessive loss of ions. To our knowledge no quantitative studies have been reported on the respiratory and nutritional demands of the brood in the ovary, nor on the capacity of the whole ovary to meet these requirements.

In this paper, weight and surface area relations between the brood and the ovary are presented for two species of Embiotocidae, the pile perch (*Rhacochilus vacca*), and the striped seaperch (*Embiotoca lateralis*). These parameters are related to the load on the ovary imposed by the requirements of the young during middle and late gestation. In addition, the morphometrics governing diffusion of materials between the ovary and the young are presented and discussed.

Materials and Methods

Adults

Observations were made on 28 pile perch (*R.* (= *Damalichthys*) *vacca*) and seven striped seaperch (*E. lateralis*). Gravid pile perch females were caught in Departure Bay, Vancouver Island, B.C., from May 20 to July 24, after which no fish were seen until August 20. Subsequently, only postparturition females could be caught. Striped seaperch were obtained from Long Beach, Vancouver Island, B.C.

Between the beginning of May and the end of August the temperature in saltwater holding tanks rose from 10 to 18 C following normal seasonal change. Fish were fed twice a week on intact mussels, a normal component of their diet.

Individual adults were taken from the holding tanks and killed instantly by a blow on the head. No anesthetics were used to avoid any side effects on the young. Each fish was blotted with a damp cloth to remove excess moisture, then weighed to the nearest gram. The ovary was exposed by a median ventral incision through the abdominal wall. The young were usually removed through an incision in the ovary wall. In most cases this operation was performed in a darkened room, as it was observed that the blood tended to drain out of the vascular paired and median fins of the young on exposure to strong light. The dry weight of the filleted carcass was determined by oven drying at 105 C for 48 hr.

Four adults were held until birth of the young had commenced. These were killed and the young examined half way through parturition, which took place over 24–48 hr.

Young

Following removal and counting of the young, a sample of 10 was taken at random. Each fish was blotted and weighed to the nearest 0.001 g. They were then oven dried at 105 C for 24 hr and reweighed. A second sample of five young was carefully laid out in a water-filled dish for photographing, with the fins and spatulate extensions at the tips extended as far as naturally possible. The magnified image (10–20×) was traced on paper. The area of the median and caudal fins and the projected area of the body were calculated.

The gills were examined during gestation and, where the secondary lamellae had commenced differentiation, the gill area was determined. The general methods of Hughes (1966) and Muir and Hughes (1969) were used, with some modification because of the small size of the gills. Each gill arch was treated separately. Those on one side were used to calculate the number of secondary lamellae differentiated on each arch. The total number of filaments with differentiated secondary lamellae was counted, and also the total number of secondary lamellae for 10% of these filaments, distributed uniformly around the gill arch. Each filament in a pair was measured separately, since they were usually of different lengths.

The gill arches on the other side of the body were used to measure the area of the secondary lamellae. Sections of the gills were mounted in an optimum freezing mixture, and transverse sections of the filaments cut on a cryostat. The outline of representative secondary lamellae for the whole length of filaments and for a uniform distribution of filaments around each arch was drawn on squared paper with the aid of a calibrated eyepiece graticule. The area of each gill arch was calculated from:

$$A = N_f \cdot \bar{N}_s \cdot \bar{A}_s$$

A is the area in cm^2, N_f the number of filaments with differentiated secondary lamellae, $\bar{N}_s$ the mean number of differentiated secondary lamellae on both sides of a filament, and $\bar{A}_s$ the mean secondary lamellar area. The area was doubled to obtain the area of both arches, and the areas of each pair of arches added to obtain the total gill area.

Ovary

The ovary was removed from each fish (in most cases after removal of the young), blotted, weighed to the nearest 0.001 g, and oven dried.

The volume of the ovarian fluid was determined for some ovaries, in which case the two anterior horns containing arteries and veins, and the oviduct leading to the gonopore, were held in artery clamps and the whole ovary removed intact to a tared beaker. Following removal of the young, the ovarian fluid was squeezed out into the beaker, weighed, and subsampled for specific gravity.

Four ovaries from gravid females (one striped seaperch and three pile perch) were removed intact and fixed in acetic formol alcohol. The three smaller ovaries were embedded in wax, and sectioned at 5 μ. The largest was embedded in celloidin, by the wet celloidin method, and sectioned at 10 μ. The transverse sections were stained in Delafield's haematoxylin and eosin, or with Mallory's triple stain.

Composite photomicrographs were constructed for each ovary section. The lengths of the internal ovarian surface, the periphery of the embryos, and the degree of overlap between them were measured by means of a calibrated cartographer's milometer. From these measurements, the area of the internal ovarian surface was estimated.

The capillary density was measured for the ovarian and embryonic surfaces from the original sections with the aid of a calibrated eyepiece micrometer. In addition, diffusion distances from the maternal capillaries to the ovarian fluid, the distance between maternal and embryonic surfaces, and the diffusion distance from the ovarian fluid to the embryonic capillaries were measured.

Results and Discussion

Gestation Period: Ovarian and Embryonic Structure

Before considering weight and structural relations for the brood and the ovary,[1] it is necessary to clarify that period of gestation to which the observations apply. The reproductive cycle of viviparous teleosts has generally been divided into five main phases for convenience in describing changes in the anatomy and histology of the ovary and the young (see Turner 1938b). These changes in embiotocids, relevant to the nutrition and maintenance of the young, are summarized in Table 1.

The structure of the young was generally that described for other species in the latter part of gestation, with the yolk sac absorbed (Plate 1). In all the embryos examined most of the body organs were well differentiated. The gut contained extraembryonic material and debris. The hind gut initially protruded slightly from the abdomen (Fig. 1 and Plate 1) but was enclosed within the abdomen by the time the young reached about 0.8 g. The median and caudal fins were expanded, highly vascularized, and terminated by vascular, spatulate extensions. The paired fins were similarly vascular, but lacked the extensions. One exception was observed in the smallest embryo examined (0.034 g) where the fins had not yet developed.

In view of the high degree of development of the young, the period of gestation to which most of the following discussion applies may be referred to as middle and late gestation. The structure of the young undergoes little change during these periods, but some clarification is needed with reference to the secondary external exchange surfaces of the vascularized fins and body. Ryder (1885), Eigenmann (1892), and Triplett (1960) have observed that the whole fin web is richly capillarized. However, there has been a tendency to ascribe all exchange of materials, other than by the gut and the gills, to the spatulate extensions of the median and caudal fins (see Turner 1952).

The most conspicuous feature of the embryos during gestation is the thin-walled venous system and thick-walled arterial system of the median fins (Fig. 1 and Plate 1). The veins anastomose at the base of the fins, outside the body proper, then traverse the musculature to join the cardinal veins. Arteries leaving the dorsal aorta divide within the body to enter the fins individually, adjacent to the fin rays. Arteries and veins are arranged alternately on either side of the fin rays. Veins usually follow the posterior edge of the spatulate extensions (Blake 1867).

Observations of the fin web of both species of perch showed that the whole fin web was capillarized as well as the spatulate extensions (Plate 2, A and B). The capillary bed of the interradial fin web was denser than that of the spatulate extensions, and contained smaller capillaries. It seems probable that larger vessels in the spatulate extensions function not only in gas exchange, but also as shunts to regulate fin-web flow. The structure of the fin web of the paired fins was similar to that of the median and caudal fins. Therefore, it appears that the whole of the expanded fin web of the median fins, in addition to the spatulate extensions and paired fins, is involved in the exchange of dissolved materials.

There is little change in the fin area during the gestation period (Fig. 1). However, the body area does change. Ryder (1885) and Triplett (1960) noted that the body surface was richly capillarized and, as such, should function in exchange in the same way as the fins. This was confirmed for embryos early in gestation (as in Fig. 1A). However, as em-

[1] "Ovary" refers only to the ovarian tissue, and excludes the young and the intraovarian fluid not contained in the stroma and epithelium.

TABLE 1. Summary of ovarian and embryonic changes during gestation in embiotocids. Based on descriptions for *Cymatogaster aggregatus* (Eigenmann 1892; Turner 1938b; Wiebe 1968b), *Taeniotoca lateralis* (Blanco 1938), *Neoditrema temmincki* (Ishii 1957), *N. ransonneti* (Igarashii 1961, 1962), *Embiotoca jacksoni* (Lagios 1965), and *E. lateralis* and *Rhacochilus vacca* (present observations). Five periods are distinguished (see text). (+ = small, ++ = developed, +++ = well developed)

Stage of gestation	Area of ovigerous folds	Functional area of young	Thickness of ovarian epithelium	Capillary density of ovary surface	Volume of fluid in ovarian tissue	Volume of ovarian fluid	Secretory activity of ovarian tissue	Notes
1) Virgin ovary	+		+	+	+	+	+	
2) Inseminated ovary	+	+	+	+	++	+	++	Embryonic yolk sac develops following fertilization, and is the first exchange surface elaborated. Regresses early.
3) Early gestation	++ to +++	+ to max.	++	++	+++	+	+++ to max.	Ovarian walls, followed by ovigerous folds, become more secretory and capillarized. Fluid in epithelium and connective tissue forms "reservoirs" or "lakes." Embryonic gut becomes functional as yolk sac atrophies. Spatulate extensions formed on median and caudal fins. Scales begin to appear and gills to differentiate.
4) Late gestation	+++ to max.	+++	+++ to max.	+++ to max.	+++ to +	++ to max.	+++ to +	Ovigerous folds penetrate gill cavity and come into close association with gills. Embryonic spatulate extensions absorbed towards end of this period.
5) Post parturition	+		+	+	+	+	+	

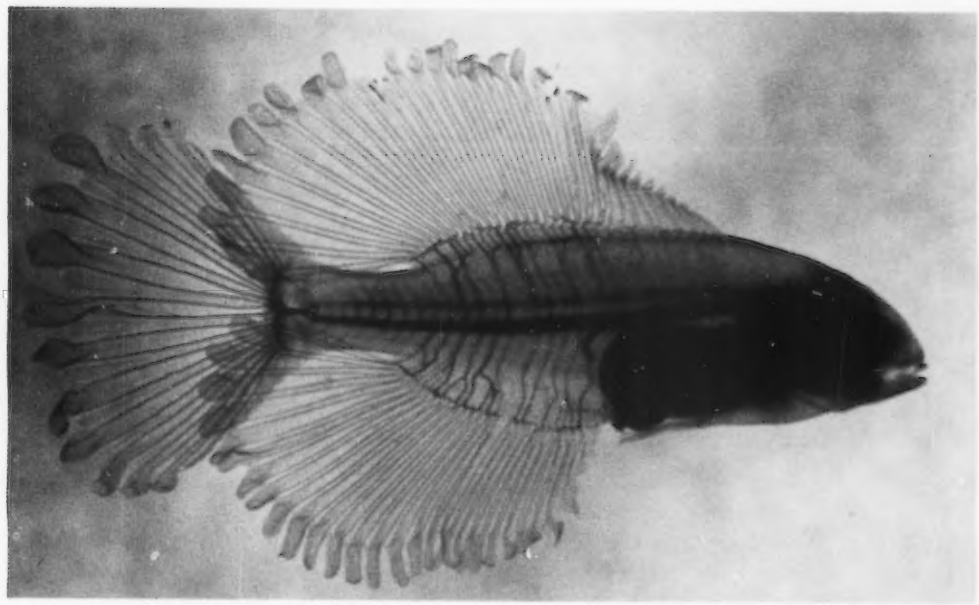

PLATE 1. Color photograph (Kodachrome II) of a prenatal striped seaperch (*Embiotoca lateralis*) (5.2 cm, 0.64 g) showing the natural appearance of the young in the middle gestation period. The fish was lightly anesthetized. The most prominent vessels (fin veins) anastomose at the base of the median and caudal fins, passing into the body to join the caudal vein. The lighter-appearing arteries leave the dorsal aorta, passing to the fins. The fin rays are transparent and cannot be distinguished. The gut, particularly the hind gut which is villified at this stage, can clearly be seen.

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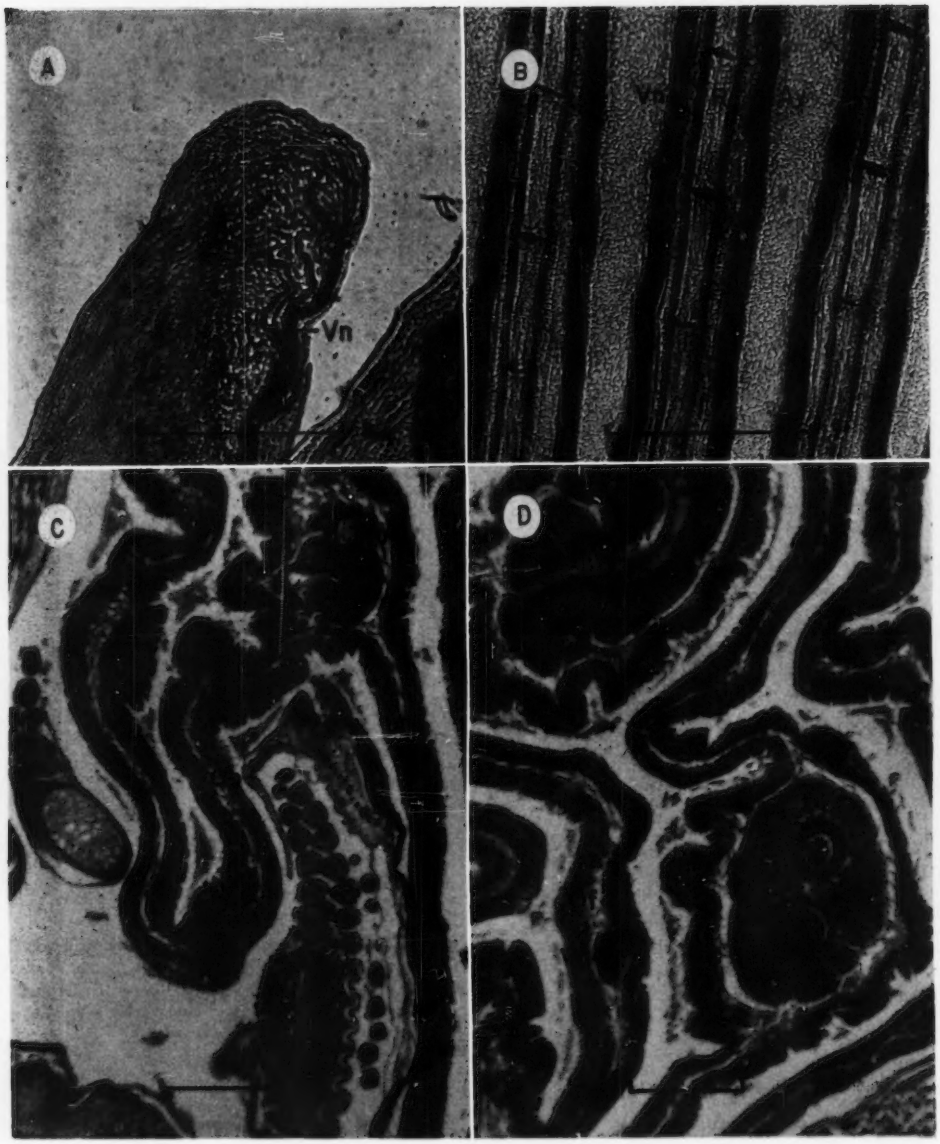

PLATE 2. Photomicrographs of pile perch (*Rhacochilus vacca*) fin and sections of ovary with young. Horizontal bars represent 1 mm in A and B, 250 μ in C and D. A) Five-second exposure of the spatulate fin extension of an intact embryo. The posteriorly located peripheral vein and network of large capillaries can be seen; B) Five-second exposure of the inter-radial fin web of an intact embryo. The streaks between the main blood vessels indicate a dense bed of small capillaries. Fin rays appear like bamboo stems; C) Transverse section of an ovary to show evagination of the ovigerous fold into the gill cavity. Note that the effect of such evaginations are often offset by the bending of the opercular flap into the gill cavity; D) Transverse section of an ovary to show the structure of artery–vein pairs. The artery and vein are both thick-walled, although the artery wall is thicker than that of the vein. Artery–vein pairs are embedded in connective tissue, but single veins are not. Key: Ay, artery; Ct, connective tissue; Gl, gill; Ofd, ovigerous fold; Op, opercular flap; R, fin ray; Vn, vein.

bryos increased in weight from 0.8 to 2.1 g, scales developed. This was associated with the disappearance of the body subcutaneous capillaries, and at the same time the body became opaque (Fig. 1B). As such, the body surface area must cease to be an exchange surface in embryos weighing more than 2.1 g.

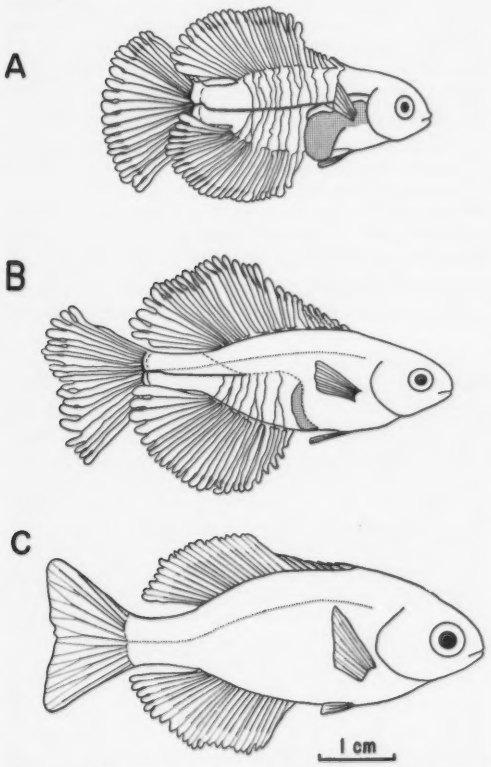

FIG. 1. Drawings taken from photographs, illustrating structural changes of the young during gestation. A) Striped seaperch (*Embiotoca lateralis*) (0.64 g) in the middle gestation period, showing the extensive venous system particularly of the fins and spatulate extensions. The arterial system and fin rays have been omitted. The gut, shown stippled, still protrudes at this stage; B) Late gestation pile perch (*Rhacochilus vacca*) (1.43 g). The dotted line crossing the body indicates the limit of scale differentiation rendering the anterior portion of the body opaque. The veins in the fins are still prominent, also the spatulate fin extensions are still present. Fin arteries and rays are omitted; C) Pile perch (*R. vacca*) young just after birth (4.41 g). The body is completely covered by scales, and vessels in the fins greatly reduced. The fin rays can be more clearly seen as shown in the drawing. The spatulate extensions of the median and caudal fins have largely been absorbed.

The external features of a young pile perch at term are shown in Fig. 1C. Prior to birth, spatulate extensions were absorbed, as observed in other embiotocids (Turner 1938b; Ishii 1957; Igarashii 1962; Wiebe 1968b). Otherwise, the bulk of the fin area remained unaltered. However, a rapid redistribution of blood apparently occurs just at birth, since the supply to the fins was progressively curtailed. At the moment of birth, the hypertrophied fin vessels contained no blood. Unborn fish from the same parent were observed to have a fully developed fin vascular system as at other stages in gestation.

WEIGHT RELATIONS FOR THE YOUNG AND COMPLETE OVARY

Brood size and growth — The brood size was correlated with the weight of the mother ($r = 0.50$; $n = 23$) but the r^2 value indicates that other factors were more important (Fig. 2). A 600-g mother could be expected to have between 30 and 50 young (mean about 42) and a 1200-g mother, between 45 and 80 (mean about 65). A similar relation between brood size and weight of the mother has been found for other embiotocids; *Amphistichus argentus* (Triplett 1960; Carlisle et al. 1960), *Cymatogaster aggregata* (Eigenmann 1892; Weibe 1968b; Wilson and Milleman 1969), and *Micrometrus aurora* (Hubbs 1921).

In a few fish (13%), dead embryos were found. These were at the same stage of development

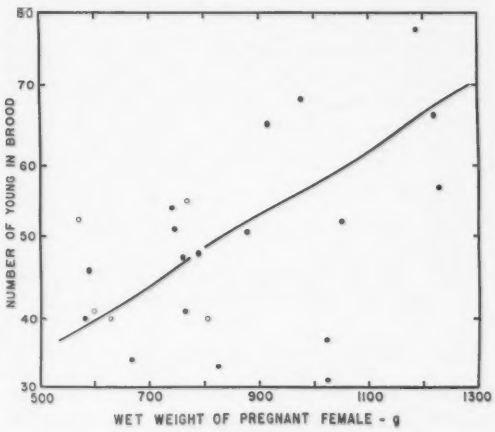

FIG. 2. The relation between the number of young in a brood and the total wet weight of the pregnant females. Solid circles — pile perch (*Rhacochilus vacca*) open circles — striped seaperch (*Embiotoca lateralis*). The line was fitted by eye.

as the remainder of the brood, with the exception of a single dead embryo found at a very much earlier stage. There was no observable relation between the dead fish and their position in the ovary.

The increase in weight of the young during gestation is shown in Fig. 3. Since the growth rate for the two species of perch was apparently different, least squares linear regressions were calculated for each species relating log W (weight of young in g) and t (time in days). For *E. lateralis* ($n = 5$) the relation was:

$$\log_{10} W = -1.7178 + 0.0093\ t \qquad (1)$$

For *R. vacca* ($n = 18$)

$$\log_{10} W = -2.9031 + 0.0226\ t \qquad (2)$$

The specific growth rates were determined as 2.05 and 4.98% wet weight/day for the striped seaperch and pile perch, respectively. Data from Wilson and Millemann (1969) gave a growth rate of 2.6% wet weight/day for *C. aggregata*, collected at bi-weekly intervals from the field.

For the progeny of the two striped seaperch and two pile perch held until parturition, the mean weight at birth was 3.84 g.

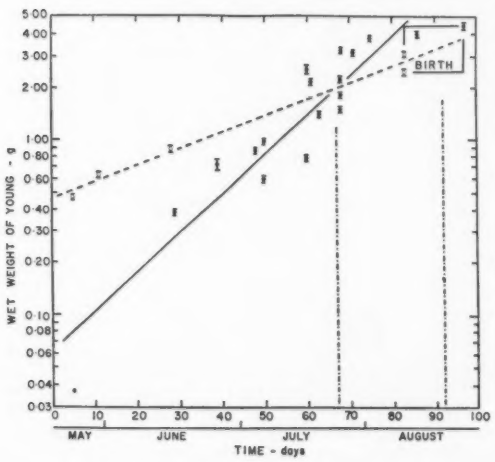

FIG. 3. Relation between the mean wet weight ($\bar{X} \pm 2SE$; $n = 10$) of the young of different parents and seasonal time (middle and late gestation). Lines were drawn from regression equations for pile perch (*R. vacca*) (solid) and striped seaperch (*E. lateralis*) (dotted). The box indicates the *weight* × *time* area of the figure in which parturition occurred in captivity. Vertical chain dotted lines indicate the period during which no wild fish could be caught by bait fishing inshore, and during which parturition must have occurred in the wild.

Captive fish gave birth between August 11 and 25. This included the latter part of the period when no wild fish were found, and during which parturition occurred in the field. This suggests that the rate of development of the captive fish was similar to that of the wild fish, and that the growth rates obtained for the former were indicative of normal growth. Further support for this is given by observations made by Wiebe (1968a), who found that the development rate of prenatal *C. aggregata* was related mainly to temperature. The perch used here were held at temperatures close to those of waters usually inhabited by the fish.

Since the variance in the data relating the weight of the young to time is fairly high, and since metabolic rates and nutritional requirements are related to weight, it is appropriate to consider the various relations between the young and the ovary in relation to weight rather than time. The weight and structural relations to be discussed below can be related to time by means of equations (1) and (2).

Dry weight and moisture content of the young — No difference was found between the two species of perch (Fig. 4). During the gestation period, dry weight increased by approximately 17 times. Since the yolk sac had already been absorbed, most of the materials for growth must have been provided by the mother, and possibly to a much lesser extent from degenerating eggs and sperm (Eigenmann 1892; Turner 1938b). The ovarian fluid contains food materials (Blake 1867) and complex materials (antibodies associated with immunological reactions)

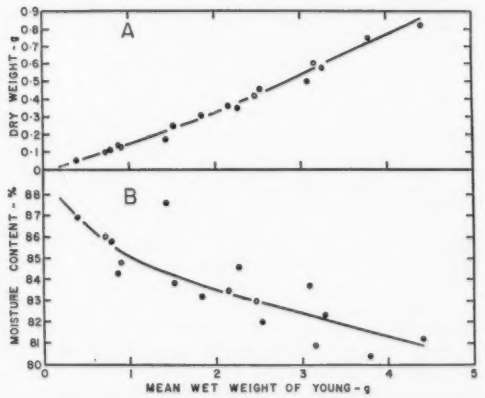

FIG. 4. Changes in dry weight (A) and percentage moisture content (B) of the young in relation to their mean wet weight. Solid circles—pile perch (*Rhacochilus vacca*); open circles—striped seaperch (*Embiotoca lateralis*).

that are transferred from the mother to the young (Triplett and Barrymore 1960a). Desquamated ovarian epithelial cells and debris have been reported in the gut of the young (Eigenmann 1892; Turner 1938b; Igarashii 1961). This was confirmed for the pile perch and the striped seaperch.

The dry weight of the adults had a mean value of 23.7% of the wet weight, a ratio which did not change during gestation.

The greatest change in the percentage moisture of the young occurred in the wet weight range from 0.5 to 2 g, coinciding with the time when the fins reached the greatest degree of elaboration (Fig. 8). A large proportion of the total moisture is undoubtedly contained in the blood supply to the fins. When the young reached about 1 g, the body increased in size while the fins did not. Therefore, the changes in the percentage moisture reflect the relative increase in body dry weight. There was no rapid change in the percentage moisture at parturition, which suggests that there was no change in body/fin ratio at that time.

Brood/ovary weight ratio — The number of young in the brood, their weight, and the weight of the ovary are all interrelated with such factors as time, temperature (Wiebe 1968a), and maternal weight. There is a slight correlation between brood size and maternal weight for both perch; a better correlation can be shown between the weight of the ovary and mean weight of the young ($r = 0.74$; $n = 21$). In general, however, it is difficult to find meaningful physiological relations between the brood and the parent because of natural variability.

The immediate environment of the young is the ovary, and conditions within it will depend on the capacity of the ovary to meet the demands of the brood. As a first approximation, the capacity of the ovary to supply these demands will depend on its weight and, similarly, the requirements of the brood will depend on the total weight of the young. Therefore, weight relations between the brood and the ovary may be expressed as a brood/ovary weight ratio, i.e.,

$$\frac{\text{total wet weight of the viable young}}{\text{wet weight of the ovary}}$$

In early pregnancy, the brood/ovary weight ratio tended toward zero, as the weight of the brood was negligible (Fig. 5). For *R. vacca*, the brood/ovary weight ratio increased to reach a plateau with a maximum value of about eight at the end of the gestation period. The few values obtained for *E. lateralis* indicate a maximum value of 13 at parturition (Fig. 5). High values for the brood/ovary weight ratio, implying a high load on the ovary in meeting the demands of the young, may explain the difference in growth rate between striped seaperch and pile perch young.

Changes in the dry weight of the ovary — A change in the composition of the ovary which is likely to be related to the problem of supply of materials to the brood is the change in the percentage moisture of the ovary, reflecting changes in ovarian fluid shifts and vascularization. This is shown as a function of the brood/ovary weight ratio in Fig. 6. It was found that there was an increase in the moisture content

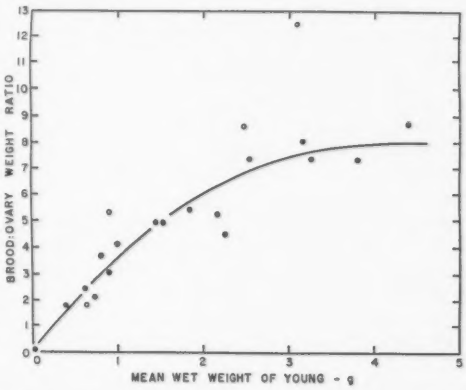

FIG. 5. The relation between the brood/ovary weight ratio and the mean wet weight of the young. Key as for FIG. 4.

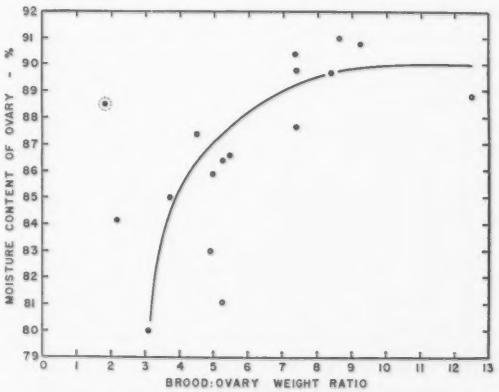

FIG. 6. The relation between the percentage moisture content of the ovary and brood/ovary weight ratio. Key as for FIG. 4. The ringed value for moisture content may reflect the storage of large quantities of ovarian fluid in the ovarian tissues during early gestation.

of the ovary from approximately 80 to 90% during the gestation period.

The accumulation of ovarian fluid in ovarian tissues early in gestation would be expected to result in a high moisture content, decreasing with the ovarian fluid shift to the ovarian cavity (Table 1). Subsequently, an increased moisture content would be expected with increased vascularization later in gestation. The relation between ovarian moisture content and brood/ovary weight ratio would thus be expected to have two maxima, the latter being shown in Fig. 6.

Changes in the volume of ovarian fluid — The shift in the distribution of the ovarian fluid from the ovarian tissue to the ovarian cavity (described by Eigenmann 1892, Turner 1938b, and Wiebe 1968b) should result in an increase in the volume of ovarian fluid. The relation between this volume and the brood/ovary ratio is shown in Fig. 7. Early in the gestation period, the volume was of the order of 15 ml.

As development proceeded, the volume of ovarian fluid increased exponentially to reach a volume of about 60 ml at the end of the gestation period, when the brood/ovary weight ratio reached about eight. This is obviously much greater than could have been originally contained within the ovarian tissues.

Eigenmann (1892), Turner (1938b), Igarashii (1961), and Wiebe (1968b) consider that secretory activity of ovarian tissue was reduced or ceased during late gestation. If this is the case, then it is difficult to explain the large increase in ovarian fluid volume. In several species of embiotocids, Triplett and Barrymore (1960b) found that the osmotic pressure of the maternal blood was greater than that of the embryonic blood. It is probable that the osmotic pressure of the ovarian fluid is intermediate. A similar difference in osmotic pressure is found between maternal and foetal blood in mammals (Hugget and Hammond 1952). The osmotic pressure gradient between maternal and embryonic blood would tend to hold water in the maternal system, rather than permit large fluid accumulations in the ovary, although this could be modified by high blood pressures in the ovarian system. The fluid could not be taken in through the genital opening as the young are stenohaline within a salinity range of 15–35% sea water (Triplett and Barrymore 1960b). Therefore, it seems probable that the accumulation of ovarian fluid within the ovarian cavity is the result of continued secretion throughout the gestation period.

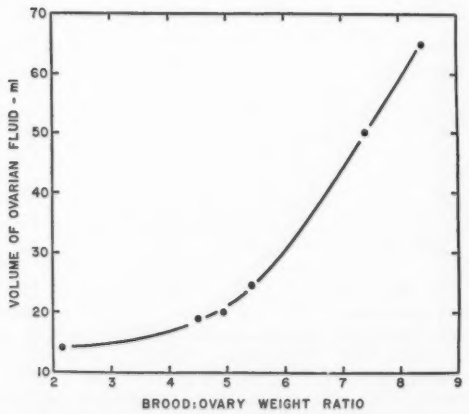

FIG. 7. The increase in volume of fluid in the ovarian cavity with increasing brood/ovary weight ratio during gestation. Values for pile perch (*R. vacca*).

MORPHOMETRICS: EMBRYONIC EXCHANGE SURFACES

In discussing the morphometrics of the young, emphasis will be placed on those surfaces involved in the exchange of soluble materials, particularly for respiration. As such, there are three main exchange surfaces: the gills, the median and caudal fins, and the body (Fig. 8).

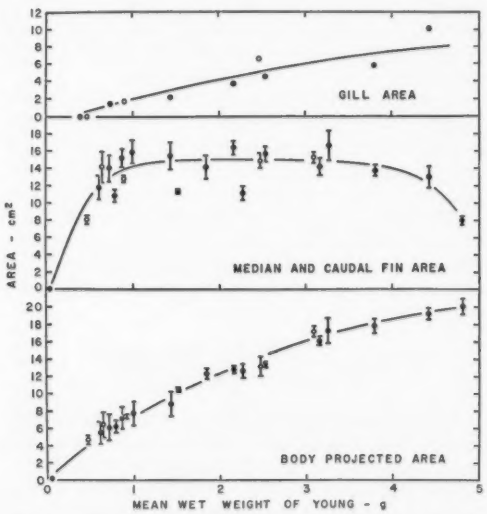

FIG. 8. The respiratory exchange areas of the young in relation to their mean wet weight. Key as for FIG. 4. $\bar{X} \pm 2$ SE; $n = 5$ are shown, except for gill area where only single fish were measured from each brood.

Gill area — In the youngest embryos examined, weighing 0.389 and 0.472 g, the gills were only partly differentiated. The majority of filaments were differentiated, and contained a central artery and vein, but secondary lamellae were not differentiated. Since the filament diffusion distance was large in comparison with those of other embryonic exchange surfaces, the gills were assumed to be nonfunctional at this stage.

Secondary lamellar differentiation had commenced by the time embryos weighed 0.7 g. They were then semicircular in profile and contained erythrocytes. Differentiation commenced at the base of each filament; as differentiation continued, the area increased and the profile became triangular.

Gill area increased from zero at 0.5 g to 7.4 cm^2 at 3.8 g, the mean weight of young at parturition (Fig. 8). The increase in area was achieved by a two to fourfold increase in secondary lamellar area (in relation to that of the first differentiated lamellae observed), a two to threefold increase in the number of differentiated secondary lamellae, and a small increase of about 8% in the number of filaments with differentiated secondary lamellae. Much of this variation resulted from differences between the gill arches. For example, the contribution made to the total area by each arch varied, the areas of arches 1 to 4 being 3.36, 4.22, 1.64, and 0.92 cm^2, respectively, for the pile perch weighing 4.4 g.

The mean gill area of the two species of perch coincides with the predicted minimum value for fish of this size, as determined from Muir (1969). Therefore, the prenatal young have apparently not elaborated their gill surface area in response to an oxygen-poor ovarian environment.

The gills probably function passively in the same way as the remainder of the exchange surfaces, rather than in conjunction with muscular branchial pumps. Observations on the frequency of opercular and buccal movements of young in the ovary, when viewed in dim red light, showed frequencies of 4–10/hr. This low rate was unchanged in fish held in one-third sea water and viewed in dim light.

Some physiological and structural factors also mitigate against the use of branchial ventilation. Efficient usage of branchial pumps, with low perfusion costs, depends largely on maintenance of a high oxygen tension gradient across the secondary lamellar surfaces (see Steen 1971). Since the ovarian-fluid oxygen tension is low (Webb and Brett 1972), this is not possible. In addition, ovigerous folds become inserted within a gill cavity of about half the embryos as in other embiotocids (Turner 1938a, 1952). This constitutes a physical block to flow across the gills, as shown for pile perch young in Plate 2C.

Median and caudal fins — The area of the median and caudal fins increased rapidly in the weight range 0.03–1.5 g, reaching a maximum value of the order of 15 cm^2 (Fig. 8). The area then remained fairly constant throughout the remainder of the gestation period until just before parturition, when there was some absorption of the spatulate extensions. At birth, the area was still of the order of 14 cm^2. After birth, atrophy of the median fin area continued until the fins reached normal adult proportions. The area of the caudal fin was of adult proportions at birth.

The body and paired fins — In all cases the projected body surface area was measured. The body tended to be fairly flat throughout the gestation period, only thickening towards parturition, as in *Neoditrema ransonneti* (Igarashii 1962). Therefore, the difference between the projected surface area and the absolute body surface area was assumed to be negligible. It was found that surface area was related to $\text{weight}^{0.63}$.

Throughout the gestation period, the pectoral and pelvic fins were pressed against the body. As a result, the area of the body beneath the fins would not be expected to contribute to the exchange area during that period when the body is vascularized. Paired fins remained vascularized after the body was covered by scales, but their contribution to the total area must remain small since only one side could be functional; they represented no more than 4% of the total exchange area.

Total and effective exchange areas of the young — The relative contributions of the gills, median and caudal fins, and body to the total and effective exchange areas are shown in Fig. 9. The small area of the paired fins is not included. The total area was calculated from the three curves in Fig. 8, being effective areas up to a weight of 0.8 g. For young of greater weight, the effective area of the body was calculated from estimates of the proportion of the body covered by scales up to 2.1 g.

The maximum effective area of 25 cm^2 was reached when the young weighed 1.5 g. Up to this weight, there was an increase in the effective body area, for although the body became progressively covered by scales, it also increased in total area. In addition, the initial decrease in the proportion of the body available for exchange was partially offset by the commencement of differentiation of secondary lamellae and functional gill area.

As the weight of the young increased beyond 1.5 g, the effective area decreased as the body progressively scaled, while gill area did not increase to fully compensate. Thus, the exchange area reached a low value of 19 cm^2 at 2.1 g on the completion of scale

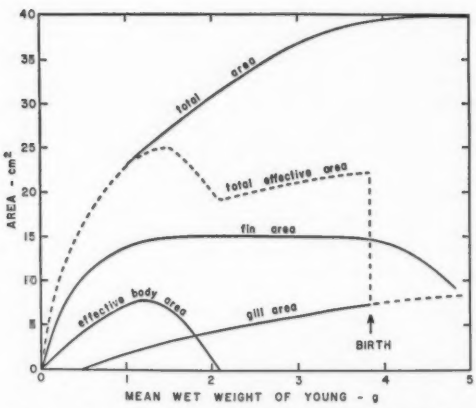

FIG. 9. Interrelations between the various areas of the young, and mean wet weight. The total area is the sum of the areas in FIG. 8. The effective body exchange area was estimated from the total area shown in FIG. 8, and the rate at which the body became covered with scales between 1.0 and 2.1 g. The total effective area is the sum of the gill area, fin area, and effective body area.

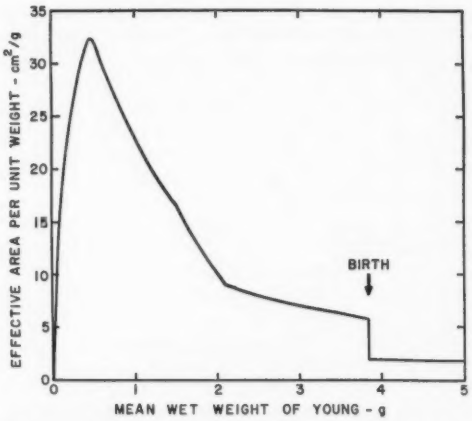

FIG. 10. Relation between effective exchange area per unit weight and mean wet weight of the young. At birth, the drainage of blood from the fins reduces the effective exchange area to that of the gills.

formation. Since there was no further change in the body or median and paired fin functional areas beyond this weight, the effective area again increased as gill area increased to reach a second maximum value of 22 cm² at parturition. At parturition, the fin vascular system was drained, leaving only the gill area to function, enhanced by the branchial pump.

Apart from the initial increase in the surface area of the young, the effective area remained between 19 and 25 cm² during late gestation, a period when the young increased in weight from 0.5 to a maximum of 4.4 g. Thus, the ratio of the effective area to unit weight steadily decreased during most of the gestation period (Fig. 10). A maximum ratio of area per unit weight occurred at a weight of 0.5 g. This was followed by a large decrease in the area per unit weight (with scaling of the body), which levelled off from a weight of 2.1 g to birth, as the gill area increased later in gestation. As a result, in the latter part of gestation when the weight increased from about 2 to 4 g, with parallel increases in the oxygen consumption (Webb and Brett 1972), the effective area of the young per unit weight decreased by only 20%.

EXCHANGE RELATIONS BETWEEN THE BROOD AND THE OVARY

Exchange surfaces — Local values for the exchange surface of the ovary and young were calculated from the length of the internal ovarian surface and the perimeter of the young measured on a given section. The total area of the internal ovarian surface was calculated on the basis of the ratio between the two lengths, and expected surface area of the brood. This latter was determined from the product of the mean number of young in the broods of the other fish (49) and the mean area of the young found from Fig. 8. Weights of the young were calculated by means of equations (1) and (2) for the date on which the ovaries were collected.

There was an increase in the total surface area of both the ovary and young. For the latter, the proportion of the total surface area represented by the fins decreased (as expected from Fig. 8). The internal ovarian surface area always exceeded that of the young (Table 2). Early in the gestation period, the ovigerous tissues were folded. They unfolded and increased in area only as development of the young proceeded in order to accommodate them (Fig. 11). Thus, as well as the increases in area, the ovarian and embryonic surfaces became better aligned over most of these areas.

Considering the simplest case of the transfer of materials between the ovary and brood by diffusion alone, the areas calculated are not the effective exchange areas. For the young, part of the total area is that of the body, progressively becoming scale covered. In addition, effective exchange surfaces are frequently folded or blocked (Fig. 11). As a result, the percentage of the total surface area available for exchange decreased during gestation (Table 2).

Similarly, the ovarian surface area available for exchange by diffusion alone was lower than the total area, and the percentage of the total area

TABLE 2. Local surface areas of the ovarian and embryonic surfaces in ovaries at four stages of development. The mean wet weight of the young was calculated from equations (1) and (2) for the date of sampling the ovary. Total surface areas were calculated assuming a mean of 49 young in each brood.

	Young in ovary					Ovary				
Section	Calculated mean wt (*g*)	Calculated external surface area of brood (cm^2)	Length of perimeter of young (*cm*)	Effective length of perimeter (*cm*)	Percentage effective length (%)	Length of internal ovarian surface (*cm*)	Effective length (*cm*)	Percentage effective length (%)	Calculated area of internal surface (cm^2)	Effective brood/ovary area ratio
Ovary 1 (Pile perch)										
Proximal	0.8	931	7.7	7.7	100	37.0	10.3	28	1441	0.74
Medial			36.7	27.7	76	68.4	34.2	50		0.81
Medial-distal			22.8	9.6	42	48.4	12.9	27		0.74
Distal			21.2	10.9	51	38.2	14.2	37		0.77
Ovary 2 (Striped seaperch)										
Medial	1.5	1225	22.3	10.6	48	30.5	12.5	41	1441	0.85
Ovary 3 (Pile perch)										
Proximal	2.5	1445	61.0	18.3	30	75.9	23.5	31	1927	0.78
Distal			57.4	17.2	30	82.5	23.9	29		0.72
Ovary 4 (Pile perch)										
Proximal	3.7	1607	77.1	14.0	18	115.4	22.3	19	2406	0.63
Medial			75.8	13.9	18	106.9	19.1	18		0.73
Distal			76.9	13.2	17	121.8	28.6	24		0.56

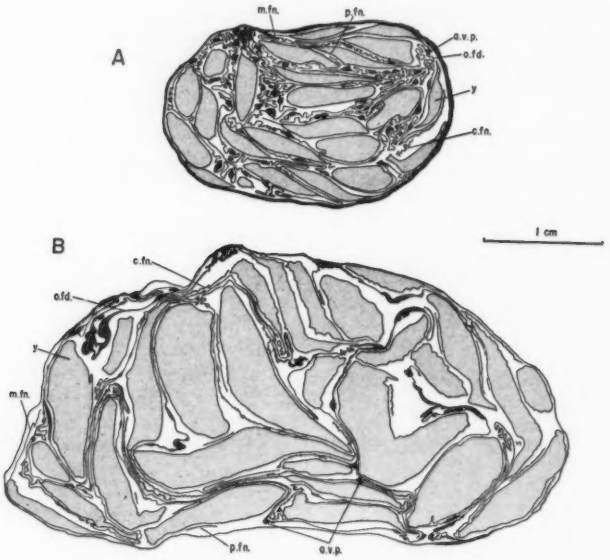

FIG. 11. Drawings, from photomicrographs, of medial sections of pile perch (*R. vacca*) ovaries in early (A) and late (B) gestation. The young are shown stippled. In A, the young had a calculated mean wet weight of 0.8 g. Note the overlapping of embryonic exchange surfaces, and the extensive folding of the ovarian tissues. The artery–vein pairs (PLATE 2D) are seen as conspicuous nodules. Fluid spaces are small. The thickness of the ovigerous folds has been reduced to show the structure of the ovary more clearly. In B, the mean wet weight of the young was calculated to be 3.7 g. Note the general absence of folding of the ovigerous folds, and the large fluid spaces. The artery–vein pair nodules are less conspicuous. Key: a.v.p., artery-vein pair; c.fn., caudal fin of young; m.fn., median fin; p.fn., paired fin; o.fd., ovigerous fold; y., young (stippled).

available decreased (Table 2). This was because of folding of the ovigerous folds in early gestation (Fig. 11A) and their alignment with the scale-covered bodies of the young in later gestation (Fig. 11B).

Those areas of the ovary and young that would be effective in diffusion alone may be expressed as an effective brood/ovary area ratio, defined in the same way as the weight ratio:

$$\text{Effective brood/ovary area ratio} = \frac{\text{effective external surface area of brood}}{\text{aligned surface area of ovary}}$$

Values for the effective brood/ovary area ratio are shown in Table 2, last column. The values decreased during the gestation period as a result of an increase in the fluid distance separating ovarian and embryonic surfaces. Thus, although the alignment of ovarian and embryonic surfaces improved, the total area of ovarian tissue required to enclose a space containing fish increased, while the fin area of the young remained fairly constant.

Diffusion distances — Maternal and embryonic diffusion distances were measured from the center of a blood capillary to the surface of the epithelium, and the fluid distance as that separating the epithelial surfaces above a capillary. These distances are included in Table 3. The embryonic diffusion distance remained unchanged during the gestation period, its mean value being 4.7μ. The ovarian diffusion distance increased from a value of 28μ in the earliest ovary examined to 41μ in the ovary examined close to term. These latter observations were consistent with the changes in ovarian epithelial height reported for *C. aggregata* for the same period of gestation (Wiebe 1968b).

The fluid distance separating effective ovarian and embryonic epithelia for diffusion exchange was very variable. During the gestation period, the mean value and range increased. As a result, the total distance separating ovarian and embryonic blood streams increased from the order of 93 to 150μ between the earliest and latest ovaries studied; most of this increase resulted from the increased fluid distance.

Capillary density — During the gestation period, the capillary density in the ovarian exchange surface increased from 28/mm to 42/mm in the earliest and latest ovaries examined (Table 3).

According to the data presented in Table 3, the capillary density of the embryonic exchange surfaces also increased. However, this was because the measurements made on the earlier young included the body surface area, which contained no capillaries close to the surface in later stages with scales. The capillary density in the body surface when vascularized was slightly lower than that of the fins. The fin surface capillary density remained constant, and the small change in the mean capillary density of the total external exchange surface may be neglected.

General Discussion

Weight and Transfer Capacity Changes During Gestation

The supply of materials for growth and respiration and the removal of wastes must be mainly by diffusion between the ovarian and embryonic blood streams. The exception to this would be some growth requirements secreted by the ovary and diffusing across external and gut surfaces of the young, and some excretory products. These could be excreted by the kidney of the young. Apart from these possible exceptions, the rate of transfer of some material, Q, can be related to the available surface area for exchange, A, and the diffusion distance, L, by means of Fick's diffusion equation:

$$Q = \Delta C \cdot k \cdot A/L \qquad (3)$$

where k is the diffusion coefficient for the material and expresses the resistance to diffusion per unit area, length, and concentration gradient; k depends on the solubility of a given material in the various layers separating ovarian and embryonic blood streams (Metcalfe et al. 1967).

ΔC is the concentration gradient, and depends on those physiological factors relating to the supply of materials by the maternal blood stream, and their rate of uptake by the embryonic blood stream. The opposite is the case for excretory products. The magnitude of ΔC for a given value of Q must reach minimum values dependent on the dimensions of the exchange surfaces. This will be discussed in greater detail in the following paper (Webb and Brett 1972) in connection with values of Q measured for oxygen transfer.

$k \cdot A/L$ represents the transfer capacity of the brood/ovary system per unit ΔC, and depends only on the morphometrics of the system as for a

Table 3. Capillary density and diffusion distances for the ovaries of Table 2.

Section	Capillary density: Ovarian surface Mean ± 2 SE (μ)	Capillary density: Embryonic surface Mean ± 2 SE (μ)	Ovarian tissue diffusion distance Mean ± 2 SE (μ)	Fluid distance separating ovarian and embryonic surfaces: Mean ± 2 SE (μ)	Fluid distance separating ovarian and embryonic surfaces: Range (μ)	Embryonic tissue diffusion distance Mean ± 2 SE (μ)
Ovary 1						
Proximal	28±6	36±4	29±3	60±13	10–275	4.5±0.9
Medial	26±7	35±2	27±3	58±14	0–250	4.9±0.9
Medial–distal	30±4	38±4	28±4	60±21	0–400	4.3±1.1
Distal	27±5	36±3	26±3	60±18	0–300	4.6±1.0
Ovary 2						
Medial	33±7	40±5	35±5	61±18	0–280	5.0±1.2
Ovary 3						
Proximal	35±8	40±3	41±3	73±44	5–400	4.2±0.7
Distal	39±8	38±3	42±3	85±51	0–380	4.9±1.0
Ovary 4						
Proximal	41±9	39±3	33±7	101±75	0–550	4.7±0.6
Medial	46±9	38±4	36±4	98±73	0–500	5.1±1.0
Distal	40±10	39±3	30±8	106±65	0–475	4.5±0.9

given material, k will be a constant. The transfer capacity can therefore be simplified to A/L for a specified substance. Changes in the transfer capacity per unit ΔC can only be brought about by structural changes. However, the rate at which a new equilibrium is reached following a change in ΔC or Q will depend on the transfer capacity (Metcalfe et al. 1967).

Physiological changes in ΔC to accommodate increased requirements of the brood would be more costly energetically than structural changes because, in the former case, higher blood flows would generally be required. In addition, the supply of the needs of the young by physiological changes cannot incorporate any safety factor should the blood be temporarily required elsewhere; for example, in a prolonged escape from a predator. Therefore, the structural changes affecting transfer capacity will be discussed in relation to the development of the young and their increasing requirements during the gestation period.

For the two species of perch, the growth rate of the intraovarian young was a constant percentage of their wet weight per day, so that the value of Q relating to growth may be expressed as a function of weight. It must be assumed that any growth materials secreted by the ovary, or taken in by the gut, remains a constant proportion of the total needs. Thus, the increase in diffusing growth materials was of the order of 17 times during the gestation period (excluding the smallest young found, weighing 0.034 g, for which no dry weight determinations were made). Similarly, metabolic rates of individual young increased by a factor of about 14 during the same period (Webb and Brett 1972). It is probable that the production of wastes is weight dependent so, for the present purposes, Q can be replaced by a function of weight, as $Q = f(W)$.

The increase in demands of the young, as f(W), were not associated with any marked increase in their effective exchange area, apart from the initial increase up to a weight of 1.5 g. On the contrary, the effective exchange area per unit weight decreased during most of the growth of the young from 0.5 g to parturition. Most of the decrease in effective area per unit weight occurred in the weight range 0.5–2.1 g, while in the 2–3.8 g range there was only a further 20% decrease. This latter increase in weight during late gestation took place over the last 20–30 days.

Although there was little change in the dimensions of the embryonic exchange surfaces during most of the intraovarian growth phase, changes did occur in the ovary that would affect the transfer capacity. As the load on the ovary increased with the brood/ovary weight ratio, there was an increase in the total ovarian surface area, in capillary density, and volume of ovarian fluid. However, if a pure diffusion system is assumed for the transfer of materials from ovarian to embryonic blood streams, then these increases were offset by the decreased proportion of ovarian surface aligned with effective embryonic surfaces, and an increased fluid distance. Furthermore, since the effective brood/ovary area ratio was always less than one, the limiting area for exchange remained that of the effective embryonic exchange surfaces.

Those factors that would operate to reduce the transfer capacity of the brood/ovary system would be largely eliminated if there were convection in the ovarian fluid. In early ovaries the volume of fluid was small, and most of it was located at some distance from the young and separated from them by folds in the ovigerous "sheets." Fluid spaces were also small. Therefore, convection would be difficult. As such, the diffusion distance would probably include the distance occupied by fluid separating the exchange surfaces, and hence would be 93 μ in the earliest ovary observed. As the young developed, there was a large increase in the size of fluid spaces around them so that the total distance separating ovarian and embryonic blood streams increased to 150 μ. However, convection would be facilitated by these larger spaces, the greater volume of ovarian fluid contained in the ovary, and the general absence of folding of the ovigerous tissue. Under convection conditions, the concentration of materials would be similar at both the ovarian and embryonic surfaces, such that the diffusion distance would be reduced to the combined thickness of the ovarian and embryonic diffusion distances; that is, 47 μ.

Although convection within the ovarian fluid would increase the transfer capacity by effectively reducing the diffusion distance, the limiting area for uptake by the young would be unchanged. However, convection could also increase the average concentration gradient between the ovarian fluid and the embryonic blood, as the whole ovarian surface could be functional in the diffusion of materials into the fluid. For a pure diffusion exchange system only a part of that surface would be functional.

In this respect, it should be noted that the structure of the ovarian blood system is apparently designed for the uniform distribution of arterial blood throughout the ovarian surface. Turner (1938b) describes "peculiar thick walled vessels" ramifying through the ovigerous folds in *C. aggregata* and branching into the capillary beds. Usually, a branch of the main artery and vein, which enter the ovary together through the two anterior horns, are seen paired and embedded in connective tissue while other individual veins are seen without such

a sheath. This structure was observed in the pile perch and striped seaperch ovaries (Plate 2D). It may be interpreted as a mechanism to reduce diffusion between arterial and venous blood entering the ovary, and between arterial blood and ovarian fluid before the capillary beds are reached. Thus, even distal capillary beds would be supplied with complete arterial blood, and no advantage would be obtained by the young in different positions in the ovary. The low variance in the weight of the young in a brood indicates that this occurs. Of all the young examined (n = 1122), only one underdeveloped, dead embryo, at a very early stage in development, was found.

Convection within the ovarian fluid requires some muscular driving force, and several possible mechanisms are present. Firstly, the musculature of the young is fully developed at an early stage, and the young can be seen to move in the ovary when this is viewed in dim red light. Secondly, the ovary wall contains some longitudinal and circular muscle (Eigenmann 1892; Turner 1938a; Wiebe 1968b) which could keep ovarian fluid in motion. In addition, normal adult behavior would tend to circulate the ovarian fluid. For example, peristaltic gut movements and body swimming movements would be transmitted to the ovary. If necessary, the muscle contained within the thin abdominal body wall could be used. Earlier in the gestation period these movements could be transmitted to the ovary through the coelomic fluid but, later, they could be transmitted directly, as the ovary occupies most of the abdominal cavity.

In one pregnant female, late in the gestation period, abdominal wall movements were observed. Several waves were seen to pass along the thin abdominal portion of the body wall only. The movements could have been the result of parental body wall muscular activity, or muscular activity of the young.

In summary, it may be concluded that the value of the diffusion distance, L, is modified by convection during the gestation period such that it decreases. The value of the area for the uptake of materials and the limiting surface for diffusion is that of the effective surface area of the young. On this basis, the relations between Q = f(W) and transfer capacity, proportional to A/L, have been summarized in Fig. 12. Mean values for W, A, 1/L, and A/L have been calculated as multiples or fractions of their values at parturition, these latter values being made equal to 1. Values for 1/L were assumed to follow a curve similar to one relating the volume of ovarian fluid to the mean wet weight of the young, with limiting values of L and 93 μ and 47 μ at weights of 0.8 and 3.7 g, respectively. It is further assumed that the value for the diffusion coefficient is constant, including those stages where the diffusion distance would include both tissue and fluid barriers.

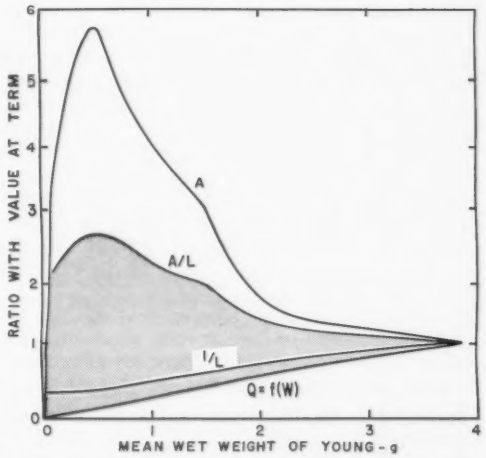

FIG. 12. Interrelations between structural parameters of the brood/ovary exchange system and the demand of the young, according to mean wet weight. The demands of the young, Q, are shown as a function of weight, f(W). A is the effective area of the young per unit weight, and L the diffusion distance. Values are shown as multiples or fractions of the conditions at parturition, which were assigned a value of 1. The stippled area shows the extent of the calculated safety factor in the structure of the brood/ovary for the diffusion of materials (in relation to the minimum that would be required to meet the requirements of the young).

The inclusion of values for 1/L in transfer capacity modifies the curve relating this to weight compared with the curve for area alone. Maximum A/L still occurs at 0.5 g, but its magnitude is reduced compared with conditions at term. The change in A/L late in gestation is also reduced to 10%, compared with 20% for area alone. Thus, convection in the ovarian fluid, together with the increase in gill area with constant fin area, interact such that there is only a small change in the transfer capacity during the period in which the weight of the young almost doubles in late gestation.

It can be seen from Fig. 12 that there is a large safety factor (shaded portion in that figure) in terms of transfer capacity during most of the gestation period. Thus, changes in the immediate demands of the young could rapidly be met with changes in the concentration gradient, particularly in early gestation. In the event of a shutdown in the ovarian blood supply in an emergency situation, the young could exist for some time on the stores contained in the ovarian fluid (Webb and Brett

1972). These would be rapidly replaced once normal conditions returned. The transfer capacity safety factor is highest in early pregnancy at that time when the young are generally least resistant to stress (Triplett and Barrymore 1960b). Later in gestation, when the transfer capacity safety factor is smaller, any failure of the supply system would be less important, as the young could be born and survive. In addition, with the high transfer capacity safety factor, given values of Q could be met by minimal values of ΔC. Since ΔC will depend mainly on the rate of blood flow in the maternal blood stream, this would make for good economy in terms of the overall energetics of the system.

In the mammalian uterus/placenta organization, it is generally considered that the supply of materials to the foetus will depend on the rate of the maternal blood flow rather than any structural limitations (Faber and Hart 1966; Metcalfe et al. 1967). In view of the large transfer capacity safety factor in the perch brood/ovary organization, a similar conclusion may be made for seaperch.

Comparative Morphology and Physiology in Viviparous Teleosts

With the exception of a few species of Goodeidae, the embiotocids are unique amongst viviparous teleosts in that large embryonic exchange surfaces are elaborated and maintained throughout gestation and for a long time after birth. In addition, the young are born in a high stage of development, young males often being mature and fertile at birth (Eigenmann 1892; Turner 1938a; Igarashii 1961, 1962).

In all but one species of Goodeidae secondary exchange surfaces develop from the anal region (trophotaeniae) but, in contrast to the fins of the Embiotocidae, these are usually absorbed just prior to birth (Turner 1933, 1937, 1940c; Mendoza 1937, 1939, 1956, 1958, 1965). However, in *Zoogeneticus quitzeoensis*, trophotaeniae are only partially absorbed at birth, being completely lost soon afterwards (Turner 1940c), while in *Girardinichthys innomatus* one pair persists (Turner 1933).

Those fish which elaborate large surfaces during their intraovarian existence must render these nonfunctional at birth. In the intraovarian environment, the composition of the ovarian fluid can be controlled by the adult, so that prenatal young are not dependent on their own ionic control systems. In the free-living existence, young are immediately dependent on their own control systems. The maintenance of large functional exchange surfaces after birth would result in a high flux of salts and water across those surfaces which would undoubtedly exceed the capacity of the young to control their ion and water balance. In the embiotocids, this problem has been resolved by shunting blood away from the fin-web capillary bed at birth. The result is that the fin surfaces cease to be functional in exchange after birth, and all gas exchange occurs via the gills with normal branchial ventilation. It must be assumed that a similar mechanism exists in those Goodeidae where the trophotaeniae are not completely absorbed at birth.

In other viviparous teleosts, primary embryonic exchange surfaces (represented by the yolk and pericardial sacs and portal blood networks) and the secondary exchange surfaces (trophotaeniae) are absorbed before birth (Turner 1933, 1936, 1937, 1938a, b, 1940a, b, c; Mendoza 1937, 1939, 1956, 1958, 1965). Those young with secondary surfaces, the Brotulidae and most Goodeidae, therefore, become dependent on ovarian adaptions close to parturition. The Jenynsidae, which have no special secondary surfaces and early absorption of primary surfaces, are dependent on such adaptions for almost the whole of the gestation period. The only known ovarian adaption is that of the invasion of the gill cavity, and in some cases the pharynx, by an evagination of the ovigerous fold to lie in close association with the gill surface (Turner 1940d, 1952; Mendoza 1956, 1958, 1965). Thus, these fish avoid the problem of ionic control after birth by an ovarian adaption. The intrusion of ovarian tissue into the gill cavities has also been described for several species of Embiotocidae (Turner 1938b, 1952; Igarashii 1962; Wiebe 1968b; and Plate 2C here). However, in these fish, it is only an accessory exchange adaption, and the fish are not entirely dependent on it as up to half the fish have "free" gills (Turner 1952).

A different situation is found in the Anablepidae and Poeciliidae where gestation is intrafollicular (Turner 1938a, 1939, 1940a, b). In early gestation, the yolk sac and pericardial sacs develop as in other fish, but these continue to expand after yolk absorption is complete and come into close association with the follicular walls. Turner (1938a, 1939, 1940a, b) has called this close association a follicular pseudoplacenta. Later in gestation, the follicular pseudoplacenta regresses, and in the Poeciliidae this is followed soon afterwards by the birth of the young. Young are born at a fairly early stage in development, but in the Anablepidae young are retained in the enlarged follicle for a longer period, and are born at a fairly advanced stage. In this latter family, absorption is taken over by the gut and the gills, which, although initially exposed to some extent, apparently function normally as parturition approaches. For efficient usage of the gill system for gas exchange, the oxygen tension in the follicular fluid would need to be fairly high

(see Steen 1971) as, otherwise, a large amount of fluid would have to be passed over the gills for each unit of oxygen taken up. This implies a more efficient system for the oxygenation of the follicular fluid in comparison with intraovarian gestation but, as no physiological data are available for other viviparous teleosts, no further conjectures can be made.

As with most other viviparous teleosts, the Poeciliidae and Anablepidae have avoided an ionic control problem after birth. However, in the Anablepidae, continued follicular existence is achieved by the use of embryonic exchange surfaces as in the Embiotocidae, except that no special secondary surfaces are elaborated. Therefore, it may be concluded that the relative independence of the young within the ovarian cavity or follicle has permitted the greatest degree of development of those young. Since the chance of survival is greater with better development at birth, it would appear that the highest degree of viviparity amongst teleosts is that of the Anablepidae and Embiotocidae. However, it appears that the limit on the size and development of the young ultimately rests with the ovary and its capacity to supply the needs of the brood.

It can be appreciated that in the evolution of fish viviparity the phenomenon of prenatal development with its specialized requirements for oxygen and nutrient supply to the young would pose physiological problems analagous to those of the live-bearing homeotherms. In a subsequent paper these relationships are examined as they pertain to prenatal respiration (Webb and Brett 1972). Greatest demand for oxygen and greatest necessity for rapid adaptation to environmental change come immediately before and at the time of birth. Respiratory and circulatory systems must rapidly adjust to this traumatic event. These changes for the seaperch are achieved by a shunt from fin dermal respiration to normal gill function, by activating the oral and buccal pumps, and by redistribution of the blood volume withdrawn from the extensive vascularization of the paired fins.

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AMOROSO, E. C. 1960. Viviparity in fishes. Symp. Zool. Soc. Lond. 1: 153–181.

BLAKE, J. E. 1867. On the nourishment of the foetus in embiotocid fishes. Proc. Calif. Acad. Nat. Sci. 3: 314–317.

BLANCO, G. J. 1938. Early life history of the viviparous perch *Taeniotoca lateralis*, Agassiz. Philippine J. Sci. 67: 379–389.

CARLISLE, J. G., JR., W. W. SCHOTT, AND N. J. ABRAMSON. 1960. The barred surf-perch (*Amphistichus argentus*, Agassiz) in Southern California. Fish. Bull. Sacramento 109: 1–79.

EIGENMANN, C. H. 1892. On the viviparous fishes of the Pacific coast of North America. Bull. U.S. Fish Comm. 12: 381–478.

FABER, J. J., AND F. M. HART. 1966. The rabbit placenta as an organ of diffusional exchange. Circ. Res. 19: 816–833.

HOAR, W. S. 1969. Reproduction, p. 1–59. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol. 3. Academic Press Inc., New York.

HUBBS, C. L. 1921. The ecology and life history of *Amphigonopterous aurora* and other viviparous perches of California. Biol. Bull. (Woods Hole) 40: 181–209.

HUGGET, A. S., AND J. HAMMOND. 1952. Physiology of the placenta, p. 312–397. *In* A. S. Parkes [ed.] Marshall's physiology of reproduction. Vol. 3. 3rd ed. Longmans, Green and Co., New York.

HUGHES, G. M. 1966. The dimensions of fish gills in relation to their function. J. Exp. Biol. 45: 177–197.

IGARASHII, T. 1961. Histological and cytological changes in the ovary of the viviparous teleost, *Neoditrema ransonneti*, Steindachner, during gestation. Bull. Fac. Fish. Hokkaido Univ. 12: 181–188.

1962. Morphological changes of the embryo of a viviparous teleost, *Neoditrema ransonneti* (Steindachner) during gestation. Bull. Fac. Fish. Hokkaido Univ. 13: 47–52.

ISHII, S. 1957. Seasonal changes in the ovary and testis of the viviparous teleost, *Ditrema temmincki*. Annot. Zool. Jap. 30: 204–210.

LAGIOS, M. D. 1965. Seasonal changes in the cytology of the adenohypophysis and ovaries of the black surfperch, *Embiotoca jacksoni*, a viviparous perchomorph fish. Gen. Comp. Endocrinol. 5: 207–221.

MENDOZA, G. 1937. Structural and vascular changes accompanying the respiration of the proctodeal processes after birth in the embryos of the Goodeidae, a family of viviparous fishes. J. Morphol. 61: 95–125.

1939. The reproductive cycle of the viviparous teleost, *Neotoca bilineata*, a member of the family Goodeidae. Biol. Bull. (Woods Hole) 76: 359–370.

1956. Adaptions during gestation in the viviparous cyprinodont teleost *Hubbsina turneri*. J. Morphol. 99: 73–95.

1958. The fin fold of *Goodea luipoldii*, a viviparous cyprinodont teleost. J. Morphol. 103: 539–560.

1965. The ovary and anal processes of "*Characodon*" *eiseni*, a viviparous cyprinodont teleost from Mexico. Biol. Bull. (Woods Hole) 129: 303–315.

METCALFE, J., H. BARTELS, AND W. MOLL. 1967. Gas exchange in the pregnant uterus. Physiol. Rev. 47: 782–838.

MUIR, B. S. 1969. Gill dimensions as a function of fish size. J. Fish. Res. Bd. Canada 26: 165–170.

MUIR, B. S., AND G. M. HUGHES. 1969. Gill dimensions for three species of tunny. J. Exp. Biol. 51: 271–285.

RYDER, J. A. 1885. On the development of viviparous osseus fishes, and of the Atlantic salmon. Proc. U.S. Nat. Mus. 1885: 128–162.

STEEN, J. B. 1971. Comparative physiology of respiratory mechanisms. Academic Press Inc., New York. 182 p.

TRIPLETT, E. L. 1960. Notes on the life history of the barred surf-perch, *Amphistichus argentus* and a technique for culturing embiotocid embryos. Calif. Dep. Fish Game Fish Bull. 46: 433–439.

TRIPLETT, E. L., AND S. D. BARRYMORE. 1960a. Tissue specificity in embryonic and adult *Cymatogaster aggregata* studied by scale transplantation. Biol. Bull. (Woods Hole) 118: 463–471.

1960b. Some aspects of osmoregulation in embryonic and adult *Cymatogaster aggregata* and other embiotocid fishes. Biol. Bull. (Woods Hole) 118: 472–478.

TURNER, C. L. 1933. Viviparity superimposed upon ovo-viviparity in the family Goodeidae, a family of cyprinodont fishes of the Mexican plateau. J. Morphol. 55: 207–251.

1936. The absorptive processes in the embryos of *Parabrotula dentients*, a viviparous deep-sea brotulid fish. J. Morphol. 59: 313–325.

1937. The trophotaeniae of the Goodeidae, a family of viviparous cyprinodont fishes. J. Morphol. 61: 495–523.

1938a. Adaptions for viviparity in embryos and ovary of *Anableps anableps*. J. Morphol. 62: 323–348.

1938b. Histological and cytological changes in the ovary of *Cymatogaster aggregatus*. J. Morphol. 62: 351–368.

1939. The pseudoamnion, pseudochorion, pseudoplacenta and other foetal structure in viviparous cyprinodont fishes. Science 90: 42–43.

1940a. Pseudoamnion, pseudochorion and follicular pseudoplacenta in poeciliid fishes. J. Morphol. 67: 59–89.

1940b. Follicular pseudoplacenta and gut modifications in anablepid fishes. J. Morphol. 67: 91–105.

1940c. Pericardial sac, trophotaeniae and alimentary tract in embryos of goodeid fishes. J. Morphol. 67: 271–289.

1940d. Adaptions for viviparity in jenynsiid fishes. J. Morphol. 67: 291–296.

1952. An accessory respiratory device in embryos of the embiotocid fish, *Cymatogaster aggregata*, during gestation. Copeia 1952: 146–147.

WEBB, P. W., AND J. R. BRETT. 1972. Oxygen consumption of embryos and parents, and oxygen transfer characteristics within the ovary of two species of viviparous seaperch, *Rhacochilus vacca* and *Embiotoca lateralis*. J. Fish. Res. Bd. Canada 29: 1543–1553.

WIEBE, J. P. 1968a. The effects of temperature and daylength on the reproductive physiology of the viviparous seaperch *Cymatogaster aggregata*, Gibbons. Can. J. Zool. 46: 1207–1219.

1968b. The reproductive cycle of the viviparous seaperch *Cymatogaster aggregata*, Gibbons. Can. J. Zool. 46: 1221–1234.

WILSON, D. C., AND R. E. MILLEMAN. 1969. Relationships of female age and size to embryonic number and size in the shiner perch, *Cymatogaster aggregata*. J. Fish. Res. Bd. Canada 26: 2339–2344.

Oxygen Consumption of Embryos and Parents, and Oxygen Transfer Characteristics Within the Ovary of Two Species of Viviparous Seaperch, *Rhacochilus vacca* and *Embiotoca lateralis*

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Oxygen tension and content of intraovarian fluid were measured for the viviparous pile perch, *Rhacochilus vacca*, during middle and late gestation. Oxygen tension decreased with increasing demands of the brood, reaching a minimum of 13.7 mm Hg just prior to parturition. Total oxygen content of the ovarian fluid decreased to a minimum of 22 mg O_2, rising thereafter to 54 mg O_2 at parturition as the volume of ovarian fluid increased. The in vitro oxygen consumption of the young per unit weight increased 2.5 times during gestation reaching 222 mg O_2/kg/hr at birth (mean weight 3.8 g).

Oxygen consumption of two pregnant striped seaperch, *Embiotoca lateralis*, was fairly constant at 70 mg O_2/kg/hr early in gestation, increasing later to approximately 107 mg O_2/kg/hr at parturition.

An oxygen dissociation curve was determined for ventricular blood of pregnant pile perch. The $p50$ was 12.5 mm Hg. Along with other parameters, this was used to estimate the oxygen transfer characteristics of the brood–ovary exchange system, maximum possible oxygen consumption, and ovarian blood flow rate. The capacity of the system to meet the increasing requirements of the young decreased during gestation, reaching limiting conditions at parturition. It was concluded that transfer of oxygen to the young would be controlled by, and ultimately limited by, ovarian blood flow rate. The brood–ovary system is compared with the mammalian placenta.

WEBB, P. W., AND J. R. BRETT. 1972. Oxygen consumption of embryos and parents, and oxygen transfer characteristics within the ovary of two species of viviparous seaperch, *Rhacochilus vacca* and *Embiotoca lateralis*. J. Fish. Res. Bd. Canada 29: 1543–1553.

Nous avons mesuré la pression d'oxygène et le contenu du liquide intraovarien de la perche de mer, *Rhacochilus vacca*, au milieu et à la fin de la gestation. La pression d'oxygène diminue en proportion de la demande accrue de la portée, pour atteindre un minimum de 13.7 mm Hg juste avant la parturition. La concentration d'O_2 total du liquide ovarien s'abaisse à un minimum de 22 mg O_2 et remonte ensuite en même temps qu'augmente le liquide ovarien, pour atteindre 54 mg O_2 à la parturition. La consommation d'O_2 in vitro des jeunes, par unité de poids, augmente de 2.5 fois au cours de la gestation et atteint, à la naissance, 222 mg O_2/kg/h (poids moyen de 3.8 g).

Au début de la gestation, la consommation d'O_2 de deux perches striées gravides, *Embiotoca lateralis*, est à peu près stable à 70 mg O_2/kg/h. Elle augmente par la suite jusqu'à environ 107 mg O_2/kg/h à la parturition.

Nous établissons une courbe de dissociation de l'oxygène pour le sang ventriculaire de *Rhacochilus vacca*. Le $p50$ se situe à 12.5 mm Hg. Cette particularité a été utilisée avec d'autres paramètres pour estimer le transfert d'O_2 dans l'appareil d'échange portée–ovaire. La capacité de cet appareil de répondre aux besoins croissants des petits diminue au cours de la gestation, pour atteindre une condition limitative à la parturition. Le transfert d'O_2 chez les jeunes serait donc contrôlé, et en fin de compte limité, par le taux du flot sanguin ovarien. Nous mettons en parallèle le système portée–ovaire de ces poissons avec le placenta des mammifères.

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THE comparative anatomy and histology of embryonic and ovarian structural adaptations to viviparity in teleosts have received considerable attention (Hoar 1969). For embiotocids, these adaptations have been summarized by Webb and Brett (1972) with particular reference to the supply of metabolites to the developing intraovarian young. Elaboration of highly vascularized embryonic fins greatly increases the surface area for oxygen uptake, constituting the most important single adaption in these fish.

In comparison with the anatomy, the physiology of exchange of metabolites between the ovary and prenatal young has largely been neglected. Quantitative measurements of the relation between the weight and brood–ovary morphometrics in striped seaperch (*Embiotoca lateralis*) and pile perch (*Rhacochilus vacca*) were considered in relation to the structural barrier to the diffusion of metabolites (Webb and Brett 1972). It was concluded that supply would be limited by such factors as blood flow rather than structural attributes. The purpose of the following study, therefore, was to estimate the overall oxygen exchange performance of the brood–ovary system. It was anticipated that the oxygen demand of the brood and the oxygen characteristics of the intraovarian environment would be subjected to various physiological constraints influencing oxygen transfer. In the absence of such information for other viviparous fish, the brood–ovary system has been compared with the mammalian placenta. The latter system has evolved to meet the same sort of oxygen supply problems as the brood–ovary system and, consequently, its physiology serves to emphasize the importance of limiting exchange parameters.

Materials and Methods

FISH

Experiments were performed on the same fish as used in the preceding study. Most measurements were made on pile perch, *R. vacca*, but a few are reported for striped seaperch, *E. lateralis*.

Pregnant females were dissected in a darkened room to expose the ovary and remove the young through an incision in the ovarian wall.

OVARIAN FLUID AND MATERNAL BLOOD

The ovarian fluid oxygen tension was measured at 12 C for six pile perch. Two samples were drawn from each ovary into an air-free syringe, and injected into a thermostated microcuvette containing a Radiometer (Copenhagen) oxygen electrode.

An oxygen dissociation curve was constructed for a mixed sample of ventricular blood from two pregnant pile perch. Blood was collected in heparinised syringes and equilibrated with air and nitrogen, free from carbon dioxide, at 11 C. The dissociation curve was established by the microliter methods of mixtures at the same temperature; oxygen content of the blood and of ovarian fluid was measured using microliter methods (Tucker 1967).

OXYGEN CONSUMPTION OF THE YOUNG

Two samples of 2–10 excised young were immediately transferred to two 500-ml bottles used as respirometers. Initially, air-saturated 10‰ sea water (mean isosmotic) was run through each bottle. Two 150-ml water samples were siphoned from the bottom of each bottle during the flushing for oxygen determinations using the unmodified Winkler method (American Public Health Association et al. 1965). The bottles were then sealed, wrapped completely in black polyethylene, and immersed in a temperature-controlled water bath. After 1 hr, the bottles were inverted several times to mix the water. This induced the young to swim, facilitating uniform mixing, after which oxygen content was determined for two 150-ml water samples from each bottle. The young were removed, blotted with a moist cloth, and weighed to the nearest 0.001 g. Some measurements of the oxygen consumption of the young kept in 10 ‰ sea water were made 24 hr after removal from the ovary. Temperatures were controlled at the ambient temperature of the holding tank for the adult perch. It increased during the experiment from 10 C in the middle of May to 18 C at the end of August.

The young always settled at the bottom of the bottles, as observed under dim red light. They made occasional respiratory movements of the branchial apparatus, but did not swim, except as a result of the strong stimulus on mixing the water at the end of an experiment.

OXYGEN CONSUMPTION OF PREGNANT FEMALE

Two pregnant striped seaperch were held at 15 C in 180-liter "growth–metabolism" tanks (Brett et al. 1971). Their oxygen consumption was determined by sealing the tank and measuring the decrease in oxygen content after 2–3 hr.

Results

OVARIAN OXYGEN CONCENTRATION

Ovarian fluid oxygen tension (P_{fO_2}) is indicative of the extent to which oxygen supply by the ovarian blood stream is matched with the load imposed on the ovary by the brood. Changes in P_{fO_2} during gestation are best shown as a function of the brood–ovary weight ratio, since this is a measure of the load where the brood and the ovary both increase in weight during gestation (Webb and Brett 1972).

P_{fO_2} was highest early in the gestation period (Fig. 1). Extrapolating to an embryonic weight of

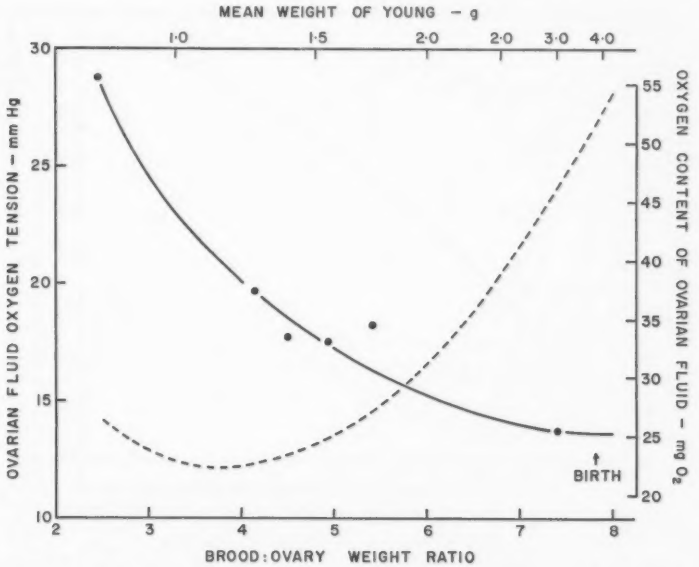

FIG. 1. Relation between the intra-ovarian oxygen environment of pile perch (*Rhacochilus vacca*) and the increasing load on the ovary, expressed as the brood: ovary weight ratio, during gestation. Ovarian fluid oxygen tension is shown by solid circles and solid line. Oxygen content of the ovarian fluid (dotted line) was calculated from this curve, the oxygen capacity, and volume of ovarian fluid (Webb and Brett 1972), which increased during gestation.

0.5 g, when structural transfer capacity was highest (Webb and Brett 1972), the peak P_{fO_2} was 36 mm Hg. The P_{fO_2} decreased exponentially as gestation proceeded, to reach a minimum value of 13.7 mm Hg towards term. Most increase in embryonic weight, from 2.0 to 3.8 g at term, occurred during the last 20–30 days of gestation. During this period, the brood–ovary weight ratio only increased from seven to eight while P_{fO_2} only decreased from 14.9 mm Hg to 13.7 mm Hg.

The ovarian fluid oxygen capacity was found to be about the same as that of 10‰ sea water, indicating the absence of any respiratory pigments. The total oxygen content of the fluid was calculated from P_{fO_2}, oxygen capacity, and ovarian fluid volumes from Webb and Brett (1972) (Fig. 1). As a result of rapidly falling P_{fO_2} as the young increased in weight, without any large increase in ovarian fluid volume, the oxygen reserve (content) decreased early in the gestation period to a minimum of 22 mg O_2 at a brood–ovary weight ratio of 3.8. Subsequently, the reserve increased towards term, reaching a maximum of 54 mg O_2, because of a rapid rise in ovarian fluid volume.

Oxygen Consumption of Young

The metabolic rate of the young measured immediately after removal from the ovary was not significantly higher than that recorded 24 hr later, permitting pooling of the data.

Oxygen consumption increased from 103 mg O_2/kg/hr for the earliest measured young at 0.389 g to a maximum of 230 mg O_2/kg/hr at 4.410 g (Fig. 2). The mean weight of the young at parturition was 3.8 g, when the oxygen consumption was 222 mg O_2/kg/hr; at 0.5 g, when structural transfer capacity was highest, the value was 105 mg O_2/kg/hr.

Oxygen Consumption of Pregnant Striped Seaperch

The metabolic rate of the parents remained constant at 70 mg O_2/kg/hr during the period of gestation when the mean weight of the young increased to approximately 1.5 g. The metabolic rate of the parents was thus about two-thirds of that of the young. As the young continued to increase in weight, the metabolic rate of the parent also increased, reaching 107 mg O_2/kg/hr at parturition, when the oxygen demand of the young

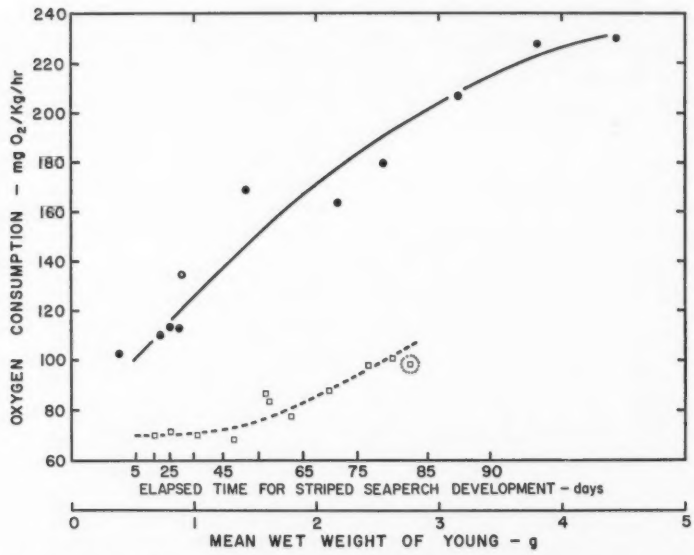

FIG. 2. Rate of oxygen consumption of prenatal pile perch (*Rhacochilus vacca*) in vitro, and of pregnant adult striped seaperch (*Embiotoca lateralis*) at normal ambient temperatures increasing from 10 to 18 C during gestation. Solid line shows oxygen consumption of young as a function of weight. Solid circles indicate values obtained for pile perch; the open circle, for striped seaperch. Open squares and dotted line, show rate of oxygen consumption of parents as a function of time. The two scales were fitted by the regression equation relating the weight of striped seaperch young during the development time from Webb and Brett (1972). The point ringed for oxygen consumption rate of adults was calculated from the minimum expected rate for the female without young, added to the expected rate of oxygen consumption of the brood.

was 192 mg O_2/kg/hr. The final total weight of the brood was 22% of the total body weight of the parents.

Maternal Blood Dissociation Curve

The oxygen dissociation curve for mixed adult ventricular blood had a $p50$ of 12.5 mm Hg (Fig. 3). The haematocrit was 25 vol.%, and the oxygen capacity 10.0 vol.% (14.3 mg O_2/100-ml blood). Because of using zero carbon dioxide levels, the dissociation curve will be most representative of arterial blood entering the ovary and for oxygen loading at the maternal gills.

Discussion

Validity of in vitro Measurements of Oxygen Consumption of Prenatal Young

Oxygen consumption was measured under conditions of temperature and darkness similar to those in the ovary. Although 10‰ sea water was used instead of ovarian fluid, this was within the range of osmotic tolerance found for shiner perch (*Cymatogaster aggregata*) embryos by Triplett and Barrymore (1960). The behavior of the young, blood distribution to external exchange surfaces, and ventilatory activity of the branchial system was typical of that in the ovary. However, metabolic rates were measured within an oxygen tension range of 100–30% air-saturation, above the range found in the ovary. Standard oxygen consumption rates of fish tend to be independent of oxygen tension in the normal ambient range, but would become dependent at oxygen tensions found in the ovarian fluid (Fry 1957).

A measure of the validity of in vitro oxygen consumption measurements can be made by comparing measured metabolic rates of pregnant adults and calculated rates based on the separate oxygen consumptions of the brood and the parent-less-brood (Table 1). The measured metabolic rate of intact

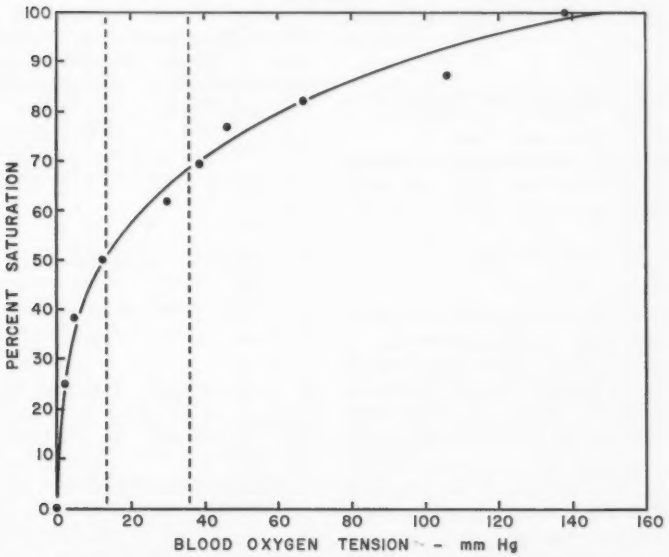

FIG. 3. Blood oxygen dissociation curve for mixed ventricular blood from two pregnant pile perch (*Rhacochilus vacca*) adults. The hematocrit was 25 vol. %, carbon dioxide tension 0 mm Hg. Vertical dotted lines indicate the range of ovarian fluid oxygen tensions expected during the gestation period.

pregnant females at term was 107 mg O_2/kg/hr, compared with a calculated value of 97 mg O_2/kg/hr. The agreement between these values may be considered good, particularly since several factors that would increase adult oxygen consumption related to support of the young are not included; for example, increased ovarian blood flow and adult gill ventilation, together with longer feeding periods and food absorption costs.

TABLE 1. Calculations for the oxygen consumption of two pregnant striped seaperch (*Embiotoca lateralis*) adults from the oxygen consumption of the brood, and the female minus the brood.

Wet Weights			
Total wet weight of pregnant female (g)	628		569
Total weight of brood (g)	99		161
Oxygen Consumption			
Oxygen consumption of brood (mg O_2/hr)	18.1		32.5
Oxygen consumption of female excluding brood (mg O_2/hr)	37.0		28.6
Mean oxygen consumption of female plus oxygen consumption of brood (mg O_2/kg/hr)		97	
Measured oxygen consumption (mg O_2/kg/hr)		107	

Vertebrate viviparity is usually accompanied by mechanisms designed to enhance oxygen supply to the young. For example, in mammals there is usually a high safety margin in structural factors, and oxygen transfer depends on placental blood flow. A similar conclusion was reached for supply of materials to the seaperch brood (Webb and Brett 1972). The blood oxygen capacity of larval and foetal blood is usually higher than that of adult blood and is saturated at very much lower oxygen tensions (Manwell 1958). In addition, a double Bohr shift operates to improve oxygen release from maternal blood, while increasing oxygen uptake by embryonic blood. The magnitude of the Bohr shift tends to be greater in young fish (Manwell 1958; M. A. Giles personal communication). Indications of the presence of similar mechanisms were noted by Eigenmann (1892) in viviparous seaperch. It is therefore likely that maternal and embryonic physiological factors would operate to meet the maximum requirements of the young up to birth.

It is therefore concluded that in vitro measurements of oxygen consumption are representative of intraovarian rates. Until intraovarian metabolic

rates are measured, in vitro rates must be considered as maximum values.

Oxygen Transfer Between the Brood and Ovary

The factors relating to the exchange of oxygen between the ovary and brood are shown by Fick's diffusion equation:

$$Q_{O_2} = \triangle P_{O_2} \cdot k \cdot A/L$$

where Q_{O_2} is the rate of mass transfer of oxygen in mg O_2/hr (per unit mass, as required). Units of mass transfer will be used rather than volume transfer, since gaseous exchange as such is not involved at the respiratory interfaces (Brett 1972). $\triangle P_{O_2}$ is the difference between mean oxygen tensions in ovarian and embryonic blood streams, k is the diffusion coefficient for oxygen, A the exchange surface area, and L the diffusion distance. These latter three factors, expressed as $k \cdot A/L$, give the structural transfer capacity as determined by Webb and Brett (1972).

Values for the factors for Fick's equation are calculated for mean brood conditions during gestation for embryonic mean weights up to 4.0 g. The mean brood size was 49 young and the mean weight of the parents, 834 g (Webb and Brett 1972).

Structural transfer capacity—The limiting exchange area, A, is that of the vascularized surface of the brood (Webb and Brett 1972). Up to a mean embryonic weight of 2.1 g, this is mainly the area of body and fins but, after that weight, only the area of fins and gills. The diffusion distance, L, is the effective distance between ovarian and embryonic blood streams. The total distance depends on convection in the ovarian fluid. Convection is unlikely in early gestation when the diffusion distance will be the combined thickness of ovarian epithelium, ovarian fluid, and embryonic epithelium. In late gestation, convection will result in similar oxygen tensions at ovarian and embryonic exchange surfaces; the diffusion distance will then be equal to the combined thickness of ovarian and embryonic epithelia. Values for A and L (Fig. 4A) were taken from Webb and Brett (1972).

Values for k have mostly been measured for nonrespiring tissue slices by Krogh (1919). The value for muscle, 0.0158 mg O_2/cm²–hr–mm Hg/μ, is likely to be most similar to the exchange epithelia. For the ovarian fluid, k would be about three times higher. However, the magnitude of the fluid diffusion barrier is difficult to assess throughout

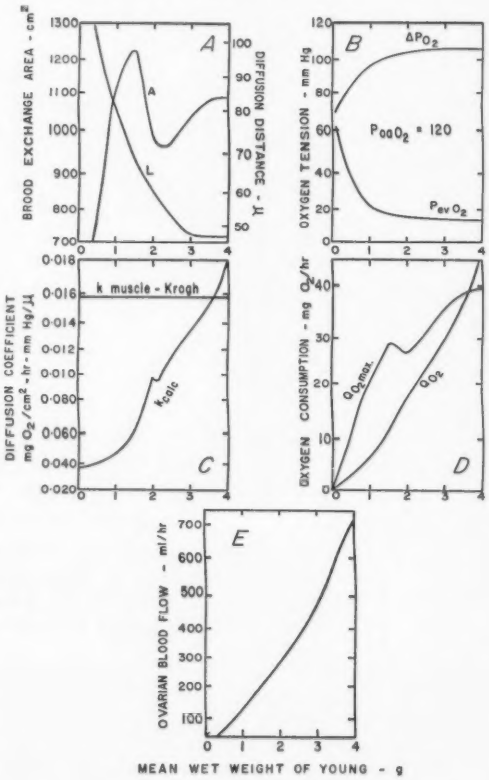

FIG. 4. Diagrams summarizing factors affecting rate of oxygen transfer in the brood–ovary exchange system, during gestation, shown as functions of the mean wet weight of the young. A) Relations between the area, A, effective for the exchange of oxygen for the brood, and the diffusion distance, L, with wet weight (Webb and Brett 1972); B) Deduced oxygen tensions, P_{aaO_2} and P_{evO_2}, respectively, and oxygen tension difference between ovarian and embryonic blood streams, assuming a perfect system; C) The oxygen diffusing capacity of the brood–ovary system, k_{calc} was calculated from Fick's diffusion equation, from data for the oxygen consumption rate of the brood, A, L, and deduced oxygen tension gradients. k_{calc} is compared with the value for muscle from Krogh (1919); D) Rate of oxygen consumption of the brood. Q_{O_2} are values measured for the brood, Q_{O_2max} are maximum values possible with the observed values of A, L, deduced oxygen tension gradients, and the muscle diffusion coefficient, calculated by means of Fick's diffusion equation; E) Calculated minimum blood flow rates required to supply the oxygen requirements of the brood, on the basis A to D, and the blood dissociation curve in FIG. 3.

gestation because of convection effects. Therefore, in subsequent calculations, Krogh's value for muscle will be used for the total diffusion distance. As a result, the magnitude of the structural transfer capacity is likely to be underestimated in early gestation, but not later, as term approaches.

It should be noted that k for live tissue could be 6 to 30 times greater than for nonrespiring tissue (Steen 1971).

Oxygen tension gradient — No measurements were made of oxygen tensions in embryonic or ovarian blood streams, but $\triangle P_{O_2}$ can be estimated from P_{fO_2} values and the published physiology of oxygen uptake in fish.

The effectiveness of blood oxygenation at the gills is of the order of 95–100% in air-saturated water, for which arterial oxygen tensions are about 120 mm Hg (Randall et al. 1967; Cameron and Davis 1970). These values are assumed for the adult pile perch. The ovarian arterial oxygen tension, P_{oaO_2}, will therefore be taken as 120 mm Hg, and the ovarian arterial blood content, 9.5 vol.% (Fig. 3).

Embryonic and ovarian blood streams exchange oxygen with the ovarian fluid; there is no particular spatial relation between the streams. The exchange system is functionally similar to the postulated "pool" system of some mammals (Metcalfe et al. 1967). Ovarian and embryonic venous blood oxygen tensions would tend to reach equilibria with P_{fO_2}. Writing these venous tensions as P_{ovO_2} and P_{evO_2}, respectively, the following applies:

$$P_{ovO_2} \longrightarrow P_{fO_2} \longleftarrow P_{evO_2}$$

The difference between P_{fO_2} and P_{evO_2} will be small because the embryonic diffusion distance is small, the exchange surface large, and well vascularized. The difference between P_{fO_2} and P_{ovO_2} will be greater than between P_{fO_2} and P_{evO_2} for, although vascularization is as good, and the exchange surface greater for the ovary than the young, the diffusion distance is also greater. However, in the following analysis, the system is assumed to be perfect with $P_{ovO_2} = P_{fO_2} = P_{evO_2}$, and $\triangle P_{O_2}$ calculated from the difference between 120 mm Hg (P_{aO_2}) and P_{fO_2} (Fig. 4B). The assumption that the difference between mean oxygen tension in ovarian and embryonic blood streams is $P_{avO_2} - P_{evO_2}$ will tend to give high values for $\triangle P_{O_2}$. However, the assumption will be offset to some extent by taking the mean embryonic blood oxygen tension equal to P_{fO_2}, and the likely double Bohr effect facilitating exchange.

Diffusion coefficient — The assumed value of k is the maximum probable value for diffusion of oxygen between embryonic and ovarian blood streams. This can be compared with the diffusion coefficient, k_{calc}, that would be required for the system to meet the oxygen requirements of the brood. Values for A, L, $\triangle P_{O_2}$ (from Fig. 4A and B), and Q_{O_2} (from Fig. 2) were used to calculate values for k_{calc} during gestation (Fig. 4C). Initially, k_{calc} was lower than Krogh's muscle value for k, but increased during gestation, reaching the same value at the stage in gestation when the mean embryonic weight was 3.6 g. The low values for k_{calc} earlier in gestation reflect the large safety factor in A/L and low oxygen demands of the brood.

Oxygen consumption — Values for A, L, $\triangle P_{O_2}$, and Krogh's value for k (Fig. 4A, B, and C) can similarly be used to calculate maximum values for oxygen consumption, Q_{O_2max}, consistent with the morphometrics and estimated oxygen tensions of the system. As expected, Q_{O_2max} was higher than the measured oxygen consumption for most of the gestation period, approaching and exceeding these latter values after the young had reached 3.6 g (Fig. 4D).

These calculations of k_{calc} and Q_{O_2max} further emphasize the hypothesis that there is a large structural safety factor early in the gestation period, and that oxygen supply to the brood will be limited by ovarian blood flow, rather than any other factor. They further suggest that the transfer limit of the system is just reached at term. The results of calculations, compared with observed values as term approaches, gives some confidence in the assumptions made.

Ovarian Blood Flow

The amount of blood required to supply the oxygen demands of the young can be estimated from assumed ovarian arterial and venous oxygen tensions, and the amount of oxygen that would be released from the blood for the oxygen tension differences. The same qualifications in assuming $P_{ovO_2} = P_{fO_2}$ must apply. Although the blood dissociation curve (Fig. 3) was determined for ventricular blood, it was purged of CO_2 and consequently values given are more likely to apply to arterial blood. The estimations of the amount of oxygen

released are therefore likely to be low under venous conditions with higher CO_2. This, however, will tend to further offset the limitations involved in the assumptions made.

The estimated ovarian blood flow increased during gestation as expected. At a stage where the mean embryonic weight was 4.0 g, the required blood flow would be highest, at 714 ml/hr (Fig. 4E), and the utilization efficiency of arterial blood oxygen would then be 46%. In reviewing several cardiovascular parameters affecting metabolism, Brett (1972) considered that a representative cardiac output for fish would be 120 ml/kg/min. Assuming no change in cardiac output during gestation, the ovarian blood flow would represent 12% of the cardiac output. This is similar to the proportion of the cardiac output supplied to the mammalian pregnant uterus (Metcalfe et al. 1967).

These calculations on blood flow in particular are at present conjectural, being based mainly on estimated exchange parameters. In addition, the oxygen demands of the ovary are neglected but, if it consumed oxygen at the same rate as the brood, and was an eighth of the weight of the brood at term, blood supply would only need to be increased to about 14% of the cardiac output. Nevertheless, the estimates of blood physiological factors affecting exchange clearly show that they will ultimately dictate Q_{O_2} for the brood, and that exchange is limited by these factors at that stage of development at which parturition occurs in nature.

Ovarian Fluid Oxygen Reserve

The oxygen contained in the ovarian fluid represents a substantial reserve that could make the brood relatively independent of maternal oxygen supply for short periods. This might be necessary should there be a temporary shut-down of the maternal system; for example, when the parent was temporarily stressed in any way. The large safety factor in the morphometrics for most of gestation would contribute to the use of such a reserve system by facilitating return to normal ovarian conditions after any shut-down period. The reserve of oxygen was lowest at 22 mg O_2 when the mean embryonic weight was about 1 g. Then, the metabolic rate of the brood would have been about 6.3 mg O_2/hr; if all the oxygen in the ovarian fluid was available to the young, it could support the brood for 200 min. A similar calculation for the brood, just prior to parturition, shows that the oxygen reserve would still support the young for a maximum of 78 min.

The ovarian fluid therefore has two main functions. It acts as a storage for brood requirements providing a buffer against temporary shortages. This buffer includes a food reserve (Blake 1867) as well as an oxygen reserve. In addition, the fluid facilitates exchange of metabolites by its convection.

Comparison of the Brood-Ovary and Mammalian Placenta

No information comparable with that for the embiotocid brood–ovary system is available for other viviparous fish; the only physiological comparisons possible are the generalizations suggested by comparative anatomy, as discussed by Webb and Brett (1972). However, the mammalian placenta is functionally similar to the brood–ovary system, posing many of the same problems. Moreover, placental exchange has been extensively studied. The comparative physiology of the two systems is therefore of value, not only as a check on the conclusions for the brood–ovary system, but also to give additional insight into the interpretation of such exchange systems and to bring emphasis to the limiting factors in oxygen exchange.

Oxygen transfer in the placenta has been summarized in detail by Metcalfe et al. (1967) and Steen (1971). The following discussion is based on these reviews. The brood–ovary and placental systems are compared in tabular form in Table 2.

The structure of the brood–ovary is most similar to an epitheliochorial placenta, in which the greatest number of tissue layers separate maternal and foetal blood streams. The blood flow pattern in the brood–ovary is functionally most similar to mammalian pool types, but in other respects blood flow patterns differ. In the placenta, some blood is shunted away from the exchange surfaces, while the brood–ovary seems to be designed for uniform distribution of all blood to the exchange surfaces. In any case, shunts in the brood–ovary would be of little use, as they would reduce the effectiveness of oxygen transfer on approaching limiting conditions at term.

A different sort of shunt is present in the brood–ovary. Some portion of nutritional requirements are shortcircuited by secretion by the ovarian epithelium, and subsequent partial absorption in the gut of the young. In fact, this nutritional function of the ovarian epithelium is probably of greatest importance, for its thickness does not provide for good oxygen transfer.

Of the factors relating to oxygen transfer, the most important difference between the two systems is the diffusion distance. For the placenta, this varies between 1 and 100 μ, compared with 33 and 450 μ in the brood–ovary. Representative distances are 3.5 μ and 47 μ, respectively, at term. The very much

TABLE 2. Comparison between structural and physiological parameters affecting the exchange of oxygen in the mammalian placenta and seaperch brood–ovary at term. Information on the mammalian placenta taken from Metcalf et al. (1967).

	Mammalian placenta	Species	Seaperch brood–ovary
Wet weight of foetus or brood at term (*gm*)	3.3×10^3	Human	1.96×10^2
		Structure	
Tissues supplied by maternal blood	Placental membrane Uterine muscle Supporting structures		Ovarian surfaces
Blood shunts outside exchange surface	Uterine and umbilical blood streams		?
Blood flow pattern	Counter-current	Rabbit	
	Multivillous	Sheep	
	Pool	Human Monkey Goat	Pool type
Tissue layers separating blood streams	Maternal capillary endothelium Connective tissue Placental epithelium	Horse	Ovarian capillary endothelium Connective tissue Ovarian epithelium Convecting fluid
	Placental epithelium Connective tissue Foetal capillary endothelium	(Epitheliochorial)	Embryonic epithelium ? Embryonic capillary endothelium
	Foetal capillary endothelium	Rabbit (Hemoendothelial)	
Diffusion distance μ			
Range.....	1–100		33–450
Representative	3.5		47
Limiting area	Foetal capillary area		Embryonic fins and gills
cm^2	1.2×10^4	Human	2.7×10^3
cm^2/gm	364		580
		Physiology	
Oxygen consumption mg O_2/100 gm-hr	63–103	Various	
	63	Human	44
Oxygen tension difference between maternal and foetal or embryonic blood streams (mm Hg) (Pool flow pattern)	20–35		106
Oxygen diffusing capacity mg O_2/hr — kg foetus or brood — mm Hg	19.7–42.9	Various	
	31.7	Human	3.6
Blood flow, percentage of cardiac output	5–20	Various	
	10	Human	12
Percentage utilization of arterial blood oxygen	28–50	Various	
	30	Human	46
Maternal blood oxygen capacity (vol. %)	15.5	"	10
Foetal blood oxygen capacity (vol. %)	20	"	?

(*Continued*)

TABLE 2. Comparison between structural and physiological parameters affecting the exchange of oxygen in the mammalian placenta and seaperch brood–ovary at term. Information on the mammalian placenta taken from Metcalf et al. (1967). — (*Concluded*)

	Mammalian placenta	Species	Seaperch brood–ovary
Maternal blood *p*50 (mm Hg) at venous *p*H.	27	"	12.5
Foetal blood *p*50 (mm Hg) at arterial *p*H.	19	"	?
Maternal hemoglobin content (gm/100 ml blood)	11.6	"	?
Foetal hemoglobin content (gm/100 ml blood)	14.9	"	?
Double Bohr shift	Present		?

greater ΔP_{O_2} in the brood–ovary reflects this difference in diffusion distance. Otherwise, differences are small. Limiting exchange area in the human foetus, for example, is a little smaller than in the brood–ovary, but oxygen consumption is higher. Blood supply (as a percentage of cardiac output) and percentage utilization of arterial blood oxygen are similar, although mammalian blood oxygen capacities are higher.

The magnitude of these differences and similarities in terms of the efficacy of the system for oxygen exchange is most clearly shown by the oxygen diffusing capacity, in mg O_2/hr-kg-mm Hg. For placentas with a pool-type blood flow, diffusing capacities range from 19.7 to 42.9 mm O_2/hr-kg-mm Hg, compared with 3.6 mg O_2/hr-kg-mm Hg in the brood–ovary. The high placental diffusing capacities show that this system would be able to adjust far more rapidly to changing demands than could the brood–ovary. However, in the latter case, changes would be temporarily met by the ovarian fluid oxygen reserve.

This, then, is the important difference. Structural improvements in placental design, reflected in the high diffusing capacity, make the system capable of meeting the requirements of the foetus throughout gestation, and always with a high safety factor. The brood–ovary only maintains a reasonable safety factor early in gestation, and none at all as term approaches.

Acknowledgments

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AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1965. Standard methods for the examination of water and wastewater. 12th ed. New York, N.Y. 769 p.

BLAKE, J. E. 1867. On the nourishment of the foetus in Embiotocid fishes. Proc. Calif. Acad. Sci. 3: 314–317.

BRETT, J. R. 1972. The metabolic demand for oxygen in fish, particularly salmonids, and a comparison with other vertebrates. Resp. Physiol. 14: 151–170.

BRETT, J. R., D. B. SUTHERLAND, AND G. D. HERITAGE. 1971. An environmental-control tank for the synchronous study of growth and metabolism of young salmon. Fish. Res. Board. Can Tech. Rep. 283: 27 p.

CAMERON, J. N., AND J. C. DAVIS. 1970. Gas exchange in rainbow trout (*Salmo gairdneri*) with varying blood oxygen capacity. J. Fish. Res. Bd. Canada 27: 1069–1085.

EIGENMANN, C. H. 1892. On the viviparous fishes of the Pacific coast of North America. Bull. U.S. Fish. Comm. 12: 381–478.

FRY, F. E. J. 1957. The aquatic respiration of fishes, p. 1–64. *In* M. E. Brown [ed.] Physiology of fishes. Vol. 1. Academic Press, Inc., New York, N.Y.

HOAR, W. S. 1969. Reproduction, p. 1–59. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol. 3. Academic Press, Inc., New York, N.Y.

KROGH, A. 1919. The rate of diffusion of gases through animal tissues with remarks on the coefficient of invasion. J. Physiol. (London) 52: 391–408.

MANWELL, C. 1958. A "fetal-maternal shift" in the ovoviviparous spiny dogfish *Squalus suckleyi* (Girard). Physiol. Zool. 31: 93–100.

METCALFE, J., H. BARTELS, AND W. MOLL. 1967. Gas exchange in the pregnant uterus. Physiol. Rev. 47: 782–838.

RANDALL, D. J., G. F. HOLETON, AND E. D. STEVENS. 1967. The exchange of oxygen and carbon dioxide across the gills of rainbow trout. J. Exp. Biol. 46: 339–348.

STEEN, J. B. 1971. Comparative physiology of respiratory mechanisms. Academic Press, Inc., New York, N.Y. 182 p.

TRIPLETT, E. L., AND S. D. BARRYMORE. 1960. Some aspects of osmoregulation in embryonic and adult *Cymatogaster aggregata* and other Embiotocid fish. Biol. Bull. (Woods Hole) 118: 472–478.

TUCKER, V. A. 1967. Method for oxygen content and dissociation curves for microleter blood samples. J. Appl. Physiol. 23: 410–414.

WEBB, P. W., AND J. R. BRETT. 1972. Respiratory adaptations of prenatal young in the ovary of two species of viviparous seaperch, *Rhacochilus vacca* and *Embiotoca lateralis*. J. Fish. Res. Bd. Canada 29: 1525–1542.

The Effects of Sublethal Concentrations of Whole Bleached Kraftmill Effluent on the Growth and Food Conversion Efficiency of Underyearling Sockeye Salmon (*Oncorhynchus nerka*)

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WEBB, P. W., AND J. R. BRETT. 1972. The effects of sublethal concentrations of whole bleached kraftmill effluent on the growth and food conversion efficiency of underyearling sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 29: 1555–1563.

Changes in growth rate and gross conversion efficiency were measured for underyearling sockeye salmon during and after exposure to full bleached kraftmill effluent (BKME). Five groups of fish were exposed for 56 days to 0, 1.0, 2.5, 10, and 25% BKME (v/v). Fresh 24-hr composite effluent at *p*H 6.8 was used daily after filtering. Growth was followed for a further 56 days after BKME exposure. Temperature was held at 15 C and dissolved oxygen between 90 and 100% air saturation. Growth rate and conversion efficiency were unaffected at 1.0 and 2.5% BKME. Reduced mean growth rate and conversion efficiency at 10% BKME were not statistically significant from controls but were considered to be biologically important in estimating threshold response levels. Significant reductions were found at 25% BKME. No recovery in retarded growth was found after exposure to BKME. Condition factor and percentage dry weight were not affected. It was concluded from computed response curves that the EC50 of the effluent was approximately 3.7% (v/v).

WEBB, P. W., AND J. R. BRETT. 1972. The effects of sublethal concentrations of whole bleached kraftmill effluent on the growth and food conversion efficiency of underyearling sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 29: 1555–1563.

Nous avons mesuré les variations du rythme de croissance et de l'efficacité de conversion brute des aliments de saumons sockeye de moins d'un an, pendant et après exposition à l'effluent entier blanchi d'une fabrique de papier kraft (BKME). Nous avons exposé cinq groupes de poissons à des concentrations de BKME de 0, 1.0, 2.5, 10 et 25% (v/v) durant 56 jours. Chaque jour, nous avons utilisé de la liqueur fraîche, recueillie sur une période de 24 h et filtrée. Nous avons suivi la croissance pendant 56 jours après exposition au BKME, et maintenu la température à 15 C et l'oxygène à 90–100% du point de saturation de l'air. Le taux de croissance et l'efficacité de conversion ne sont pas affectés par des concentrations de BKME de 1.0 et 2.5%. A une concentration de 10%, il y a diminution du rythme de croissance et de l'efficacité de conversion, statistiquement non significative par rapport aux témoins, mais suffisante pour avoir une importance biologique dans l'estimation des seuils de réponse. Il y a diminution significative à 25% de BKME. La croissance retardée à la suite de l'exposition au BKME n'est pas reprise. Le facteur d'embonpoint et le poids sec relatif ne sont pas affectés. Les courbes de réponse nous permettent de conclure que le EC50 de l'effluent est d'environ 3.7% (v/v).

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ONE of the problematic features of bioassay of industrial discharges such as pulpmill effluents is their variability both within a given plant and between plants. Howard and Walden (1971), Walden and Howard (1971), and Walden et al. (1971) sampled seven British Columbia pulp mills over 40 days and found that median survival times in full bleached kraftmill effluent (BKME) varied from 485 to 1298 min; the variance was of the same order of magnitude as the means. In addition, from 21 to 82% of samples were nonlethal in 25-hr tests.

Variability of this magnitude complicates studies of stress responses, particularly when commonly used "grabsampling" methods are employed. As is well known, such samples are not representative of long-term mill operation. Storage of effluent is

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often required resulting in deterioration which cannot be entirely eliminated (Howard and Walden 1965; Werner 1963). To overcome such problems, various physical and biological standards have been sought related to both acute and chronic stress responses. As yet no working correlations have been found between physical characteristics and acute toxicity for whole BKME (Walden et al. 1971); chemical oxygen demand (COD) of the in-plant process — streams has been used as a standard over fairly long periods for single mills only (Sprague and McLeese 1968a, b, c). It has been reported that elevated coughing frequencies are related to BKME concentration, but again for single mills only and not between mills (Schaumberg et al. 1967; Walden et al. 1970).

The most commonly used standard remains that of acute toxicity, specifically the concentration giving 50% mortality in 96 hr (96-hr LC50). This obviously excludes nonlethal toxic samples based on other criteria. Furthermore, the effectiveness of an acute toxicity standard is reduced because substantial deterioration of effluent toxicity occurs even within the test period, and fish rapidly acclimate to BKME (Howard and Walden 1965). In addition, Tokar and Owens (1968) have emphasized that the use of an acute toxicity standard to relate to sublethal effects presupposes that the different responses are caused by the same effluent components, which may not be valid.

The following experiment was therefore designed to overcome some of these problems by using fixed dilutions of fresh daily composite effluent. The sublethal responses selected were changes in growth rate and conversion efficiency since these involve relatively long-term testing and could be expected to average out much of the temporal variability in BKME. As a response mechanism, growth is a "wide spectrum" indicator sensitive to changes in appetite, digestion, metabolism, and excretion. When coupled with restricted ration it further takes into account possible elevation of maintenance metabolism which otherwise could be compensated for by increased food intake to maintain growth. The latter is detectable by determining the conversion efficiency.

Materials and Methods

Fish

Cultured sockeye salmon (*Oncorhynchus nerka*) from Pitt River, British Columbia, were acclimated to 15 C over 2 weeks, selected for uniform size, and randomly distributed to five growth tanks (60 fish per tank). Tanks were artificially illuminated for a 16-hr photoperiod; dissolved oxygen was maintained between 90 and 100% air saturation. Fish were fed at the same ration levels, starting at 15% body dry weight/day.

Growth was measured for three phases, a pre-BKME-exposure phase, a BKME-exposure phase, and a post-BKME-exposure phase. Each growth phase was arbitrarily divided into growth "periods," usually of 14 days, at which time all fish were weighed and measured under light anesthetic (to the nearest 0.01 g and 0.1 cm fork length).

Apparatus

In the pre- and post-BKME-exposure phases, five 197-liter growth tanks were used (described in Brett et al. 1969). A water flow of 182 liters/hr entered each tank after passing through a gas stripping column to remove periodic supersaturation. Temperature was maintained at 15 C by adjusting temperature-controlled supply lines. In the area of the tank preferred by the fish, the water velocity was about 18 cm/sec.

For the BKME-exposure phase, the growth tanks were converted into recirculating units. Dissolved oxygen levels were maintained by means of an air stone and temperature was maintained at 15 C by a cooling coil. Tank water velocities were kept at the same rate as above; water was also continuously recycled at 330 liters/hr through washed fiberglass filters to prevent the buildup of wastes. Twice a day, 136-liter reservoirs were filled with fresh neutralized effluent (at the prescribed concentration) which was drained via filters into the fish tanks over a 4-hr period.

BKME was obtained from a local pulp mill producing full bleached kraft pulp. Waste is discharged through two main sewers containing mainly a) the combined alkaline pulping effluents and caustic extraction bleach effluent, and b) chlorination effluent from the bleach plant, in a ratio of approximately 1:1 by volume. A 1.4-liter sample was automatically taken every 15 min from each sewer and stored separately in nontoxic containers. The daily composite sample was subsequently collected from each and mixed in equal volumes. Molar sodium hydroxide or hydrochloric acid was added as required to obtain a *p*H between 6.7 and 6.9. The *p*H of the local water to which control fish were exposed was 6.8.

Five groups of fish were exposed to fixed BKME concentrations (v/v) of 0, 1.0, 2.5, 10, and 25%. Preliminary tests had shown that the effluent was usually nontoxic in 96-hr bioassay tests. The highest concentration was selected as half the median effective concentration found to affect plasma solids (Webb and Brett unpublished data). It was also about half the 96-hr LC50 found by B.C. Research Council for five toxic effluent samples taken during the course of the experiment (T. E. Howard personal communication). The mean BOD during the exposure phase was 120 mg O_2/liter (MacMillan–Bloedel staff personal communiation).

Sudden exposure of fish to BKME results in temporary stress. As measured by coughing frequency, this persists for about 20 hr (Schaumberg et al. 1967; Walden et al. 1970). The concentration was increased to the prescribed levels over 2 days at the beginning of the BKME ex-

posure phase, and decreased over the same time at the end of that phase.

Ration and Feeding

Fish were fed twice a day at 0800 and 1600 hr on "dry" Abernathy pellets (8–10% water) except on the day they were weighed. Dry weights of food and fish were determined for each growth period by airdrying samples at 105 C for 24 hr. Initially, a restricted ration level of 15% dry body weight/day (based on dry food) was given in two equal portions per day. As the fish increased in weight, the maximum daily ration accepted showed a normal decrease (Brett 1971). The initial ration consequently approached a maximum or satiation level before the end of the experiment. Fish were then fed 5% dry weight at the first feeding and, subsequently, as much as they could take in 30 min of the remaining 10% dry weight.

Results

Specific Growth Rate and Conversion Efficiency

The slope of the relationship between the logarithm of wet weight and time was positive for all groups of fish. The slope decreased with time, as expected from the dependence of growth rate on weight. However, the exponential relation could be approximated to straight lines for each of the three growth phases (Fig. 1); this was assumed in the calculation of specific growth rates and gross conversion efficiencies (Table 1).

Pre-BKME-exposure phase — No significant differences in growth rate (Student's *t*-test; 0.05 level) developed between the five groups of fish during

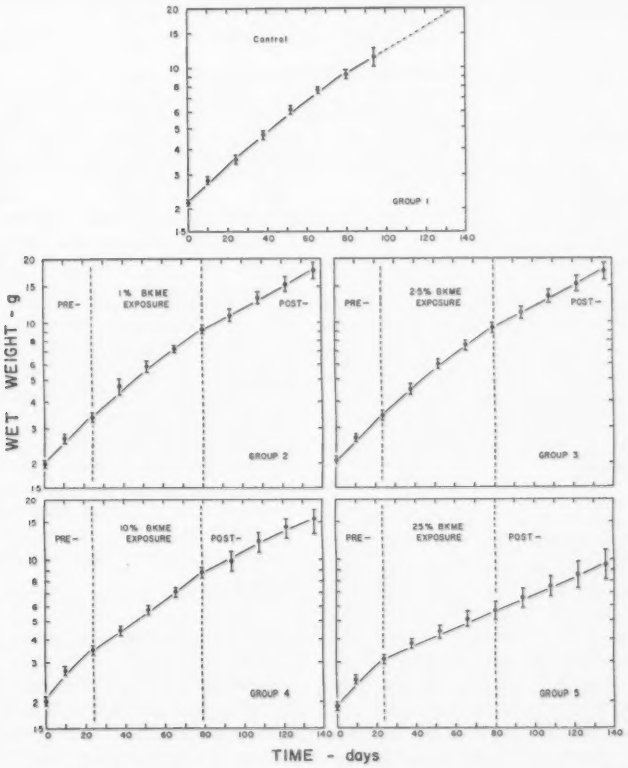

FIG. 1. Relations between wet weight ($\bar{X} \pm 2$ SE) and time for the five groups of fish. BKME concentrations (% v/v) and exposure phase are shown by vertical dotted lines. The growth rate found for control fish for the first period of the post-exposure phase is extrapolated for the remainder of that growth phase.

TABLE 1. Summarized results for growth of underyearling sockeye salmon in three growth phases. Variance, where shown, is ±2 SE, except for temperature, where ±2 SD are given.

	0% BKME	1.0% BKME	2.5% BKME	10% BKME	25% BKME
			Pre-BKME-Exposure phase		
Mean wet weight, g	2.84	2.68	2.73	2.75	2.49
Ration, dry food given as % body dry weight/day	15.1	15.1	15.7	16.6	14.2
Percent dry weight	19.7±1.1	19.7±1.5	19.7±1.1	19.5±1.2	19.8±1.6
Specific growth rate, % wet weight/day	2.21±0.26	2.30±0.34	2.18±0.32	2.33±0.34	2.09±0.32
Gross conversion efficiency, %	14.7	15.3	13.9	14.0	14.7
Temp. C	15.0±0.2	15.0±0.2	15.0±0.2	15.1±0.3	14.8±0.2
			BKME-Exposure phase		
Mean wet weight, g	6.23	6.05	6.16	5.92	4.39
Ration, % dry weight/day	14.0	14.6	14.4	14.5	14.5
Percent dry weight	20.4±1.3	20.9±1.5	20.5±1.9	21.0±1.6	20.2±1.7
Specific growth rate, % wet weight/day	1.74±0.12	1.81±0.12	1.81±0.13	1.71±0.14	1.06±0.18
Gross conversion efficiency, %	12.4	12.4	12.5	11.8	7.3
Temp. C	15.1±0.5	14.9±0.5	15.0±0.5	14.9±0.6	15.0±0.6
			Post-BKME-Exposure phase		
Mean wet weight, g	10.31	13.33	13.32	12.10	7.57
Ration, % dry weight/day	14.0	12.0	12.6	12.9	15.0
Percent dry weight	21.8±1.9	22.1±1.5	22.7±1.6	22.2±1.6	21.0±1.8
Specific growth rate, % wet weight/day	1.41±0.18	1.20±0.18	1.16±0.16	1.03±0.20	0.97±0.28
Gross conversion efficiency, %	10.1	10.0	9.2	8.0	6.5
Temp. C	14.9±0.2	14.9±0.3	14.9±0.2	14.9±0.2	14.9±0.2

this preliminary phase. Gross conversion efficiencies were also similar. The overall mean conversion efficiency was 14.5%, somewhat lower than the value of 20% found by Brett et al. (1969) for the same species at the same ration level. This difference can be attributed to higher water velocities used in the present experiment which would have raised maintenance requirements.

BKME-exposure phase — No significant differences were found for growth rate and conversion efficiency for dilutions of 0, 1.0, 2.5, and 10% BKME (0.05 level). Growth rate and conversion efficiency were significantly lower (0.01 level) for 25% BKME, the highest concentration used.

The overall mean growth rate for 0, 1.0, 2.5, and 10% BKME was 1.77%/day. Although no statistical differences were found, growth rates at 0 and 10% BKME were lower than at 1.0 and 2.5% BKME. This was because the ration was lower for 0% BKME, conversion efficiency being comparable between 0, 1.0, and 2.5% BKME. However, the lower growth rate for 10% BKME was not caused by a lower ration, but rather by lower conversion efficiency.

Post-BKME-exposure phase — The control group was terminated after 14 days (see Appendix). No significant differences were found in "recovery" growth rates between the groups of fish from all four dilutions of BKME. Nevertheless, postexposure growth rates decreased according to the BKME concentrations experienced. Conversion efficiencies also decreased in a similar fashion. For this response system, differences were not significant (0.05 level) between 1.0, 2.5, and 10% BKME, nor between 10 and 25% BKME, but were just significant (0.05 level) between 25% BKME and both 1.0 and 2.5% BKME.

CONDITION FACTOR AND PERCENTAGE DRY WEIGHT

Condition factor was typical of healthy fish throughout the three growth phases. It increased from approximately 0.0099 to 0.0104 during the course of the experiment. The increase in condition factor for 25% BKME during and after BKME exposure was lower than for other groups. However, increases were related to weight, being equal for fish of the same weight (Fig. 2). Therefore, BKME exposure had no direct effect on fish condition.

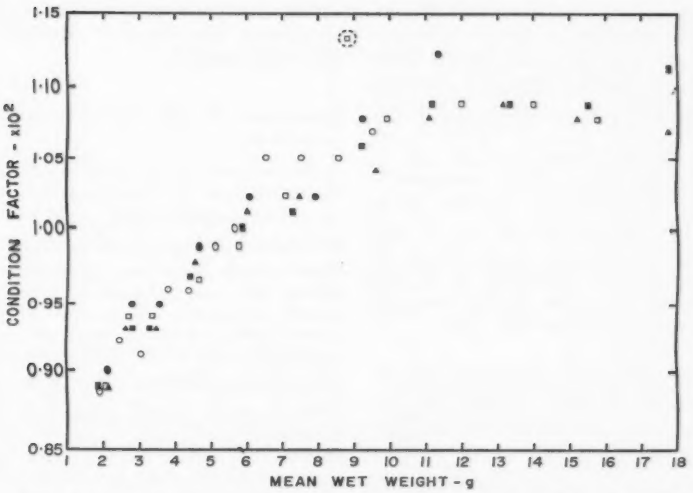

FIG. 2. The relation between condition factor and mean wet weight at various concentrations of BKME. Key: Control, solid circles; 1.0% BKME, solid squares; 2.5% BKME, solid triangles; 10% BKME, open squares; 25% BKME, open circles. The point shown circled is that for 10% BKME, measured following the death of some fish with full guts (see Appendix).

Percentage dry weight also increased slightly in all groups during the course of the experiment, a normal characteristic of growth. Increases in any one group were not significantly different (Student's t-test; > 0.05 level) between growth phases, thus permitting pooling of these data (Table 1). Average values for fish exposed to higher BKME concentration were lower than for other groups. However, these can be shown to be weight-dependent in the same way as condition factor and cannot, therefore, be attributed directly to BKME exposure.

Discussion

Comparison of Results

Because the growth response to one of the BKME concentrations (10%) appears to fall right in a transition zone, two criteria must be considered in assessing the data — statistical differences and biological sufficiency.[1] Where there are neither statistical differences nor biologically explicable differences in response between control and BKME-exposed fish, then it can be concluded that BKME had no effect on growth. This applied to 0, 1.0, and 2.5% BKME; consequently, these data can be pooled. Where there are statistically significant differences in response between control and BKME-exposed fish, the response will obviously be of biological importance. This applied to 25% BKME.

However, differences in response which are not statistically significant are not necessarily without biological importance. This appears to apply to 10% BKME. Differences in growth rate between 0, 1.0, and 2.5% BKME were caused by differences in ration level, conversion efficiencies being comparable. In contrast, reduced growth rates at 10% BKME were found to be accompanied by a reduction in conversion efficiency. During the BKME- and post-BKME-exposure phases there was a continuing trend for the weight of fish previously exposed to 10% BKME to diverge from that of 0, 1.0, and 2.5% BKME-exposed fish (Fig. 1). It is therefore deduced that the reduced growth rate and conversion efficiency at 10% BKME were real effects, of long-term biological importance.

This is typical for sublethal responses to a variety of toxicants e.g. KME (Ellis 1967; Tokar and Owens 1968), endrin (Grant and Mehrle 1970), and heavy metals (Dorfman and Whitworth 1969; Dilling and Smith 1926) and also low oxygen levels (Herrman et al. 1962; Stewart et al. 1967; Pickering 1968). In all cases, it is difficult to detect statistically significant responses near threshold levels of toxicants.

[1]Additional experiments were not possible because of changes in research priorities; hence, this had to be considered as a definitive study.

In comparing the results from the various BKME groups, the normal decrease in growth rate and conversion efficiency with increasing weight must be separated from any decrease caused by exposure to BKME. The separation was achieved as follows:

Growth rate — Curves relating growth rate to weight were calculated from weight increases in each growth period (from Fig. 1). A control curve was constructed from pooled data for 0, 1.0, and 2.5% BKME (Fig. 3A) since, as shown, growth in the latter two groups was unaffected by BKME exposure. Growth rate values for 10% BKME were all well below the control curve (Fig. 3B).

Gross conversion efficiency — Treatment of the results for conversion efficiency (in this case for each growth phase) gave a similar distribution (Fig. 4). However, the differences between the control curve and the 10 and 25% BKME curves were greater than for growth rate. This shows that conversion efficiency was a more sensitive indicator of sublethal stress.

Taken together, the curves for growth rate and conversion efficiency, as functions of weight, amplify the differences between control fish and 10 and 25% BKME. They show more clearly the biological effects of BKME on growth at 10% BKME. The curves also show that the effects of BKME were permanent, there being no recovery in the 56 days following exposure.

Threshold BKME Concentrations Affecting Growth

On the basis of the control curves (Fig. 3A and 4), response curves relating growth and conversion to BKME concentration can be constructed to better estimate mean effective concentrations (EC50) above which growth or conversion would be reduced. This can be done by expressing measured growth rates and conversion efficiencies in any growth phase as percentages of expected control values (for fish of the same weight).[2] EC50s were taken for the inflection point of the response curve, fitted to mean growth rates and conversion efficiencies (Fig. 5). The response curves for both growth rate and conversion efficiency for BKME- and post-BKME exposure phases clearly show the

[2]Percent measured/expected values tended to be greater than 100% for 0, 1.0, and 2.5% BKME because weight increase with time is exponential. Mean weight calculated from values measured every 14 days consequently tends to be higher than the true mean weight over a long period. As a result, expected growth rates and conversion efficiencies tend to be slightly low.

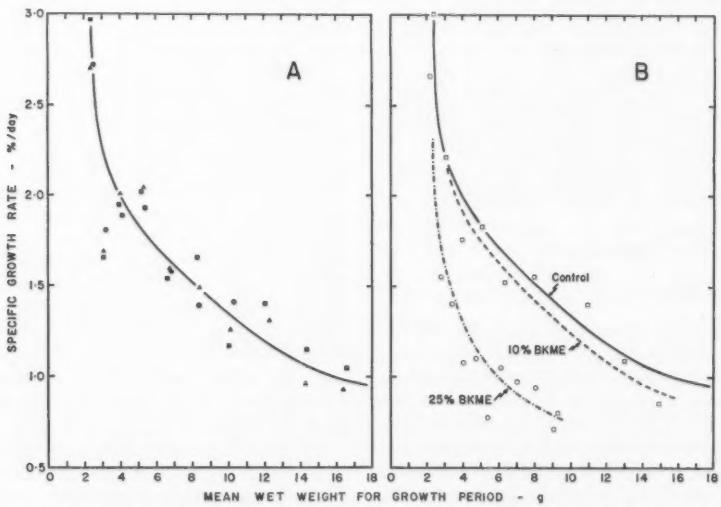

FIG. 3. The relation between specific growth rate and wet weight of fish, calculated as mean values for each growth period from data in TABLE 1. A) Shows the relation for controls, and 1.0% and 2.5% BKME unaffected by BKME. B) The relation for 10% BKME and 25% BKME shown in relation to the curve in A. Curves were fitted by eye. Key as for FIG. 2.

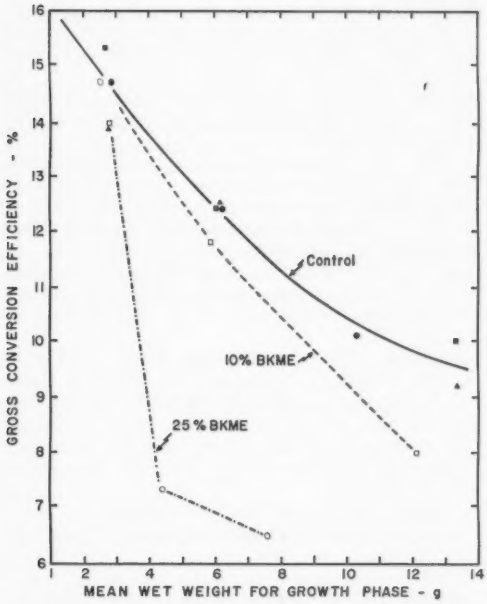

FIG. 4. The relation between gross conversion efficiency and wet weight, calculated for each growth phase from data in TABLE 1. The lines were fitted by eye. Key as for FIG. 2.

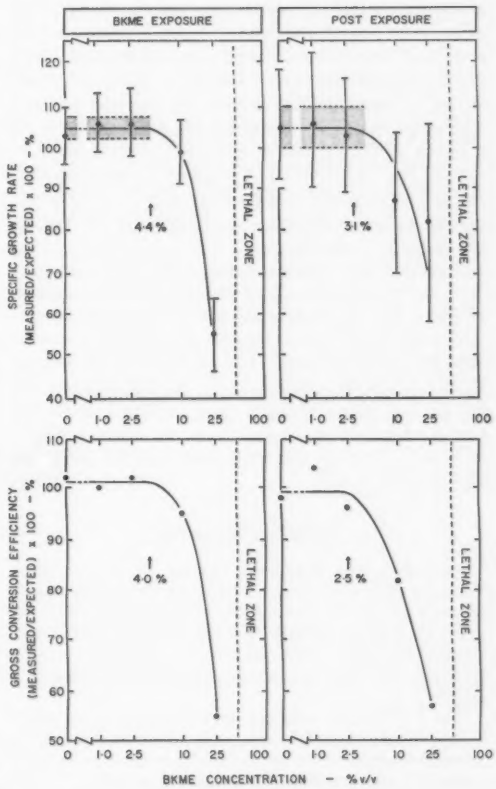

FIG. 5. Response curves showing the relation between BKME concentration and growth rate (*top*) and conversion efficiency (*bottom*) during BKME- and post-exposure phases. Measured growth rates and conversion efficiencies are shown as percentages of values expected for the mean weight of the fish in any phase but in the absence of BKME exposure, these being taken from Fig. 3A and 4. The arrows indicate inflection points and corresponding EC50 BKME concentrations. The vertical dotted lines show the 96-hr LC50 measured for five toxic BKME samples.

permanence of the effects of BKME exposure, at least in a time equivalent to the exposure time. For the BKME-exposure phase, EC50s for growth rate and conversion efficiency responses were estimated as 4.4 and 4.0% BKME, respectively, and for the post-exposure phase as 3.1 and 2.5% BKME (Fig. 5). The mean overall EC50 was 3.7%. This is 8.0% of the 96-hr LC50 measured for five toxic samples. The EC50 values suggest an increasing response with time to BKME, even after removal of the source of stress.

CAUSES OF REDUCED GROWTH AND CONVERSION EFFICIENCY

As was suggested with this form of stressor, BKME could reduce growth and conversion efficiency by reducing either one or more of food-intake, assimilation efficiency, metabolic processing, or increased maintenance requirements (Warren and Davis 1967).

No reduction in food intake was observed. Any reduction here, or in assimilation efficiency, would be equivalent to a reduced ration compared with control fish. In addition, no difference was found in percentage dry weight between groups exposed to different BKME concentrations, showing that assimilation efficiency as well as food intake was unaffected (Brett et al. 1969). The same conclusion was reached by Tokar and Owens (1968) for chinook salmon exposed to unbleached kraftmill effluent.

BKME probably raised other metabolic costs as suggested by Tokar and Owens (1968) for KME. Howard and Walden (1967) report decreased haematocrits of four salmon species exposed to BKME for 25 days; P. W. Webb (unpublished data) has found decreases in plasma solids after 5-day

exposures to BKME at concentrations of 60% v/v of a "non lethal" effluent sample. These sorts of responses would probably be associated with elevated maintenance metabolic rates. A preliminary study in this laboratory has shown that standard oxygen consumption may be elevated by a factor of about two in effluent concentrations of 1% of the 24-hr LC50. Therefore, it appears that BKME stress is associated with raised maintenance energy costs, reducing the proportion of energy available for growth.

Some of the responses noted above could also explain the permanence of BKME exposure effects. Fujiya (1961) has noted extensive damage to several vital organs of *Sparus macrocephalus* caged downstream of a mill outfall for 24 hr. Such long-term effects of BKME could be of great importance, for failure to assess them would underestimate the subtle, deleterious consequences to salmon populations long after the immediate source of stress had ceased to act.

Acknowledgments

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BRETT, J. R. 1971. Satiation time, appetite, and maximum food intake of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 28: 409–415.

BRETT, J. R., J. E. SHELBOURN, AND C. T. SHOOP. 1969. Growth rate and body composition of fingerling sockeye salmon (*Oncorhynchus nerka*) in relation to temperature and ration size. J. Fish. Res. Bd. Canada 26: 2363–2394.

DILLING, W. J., AND W. C. SMITH. 1926. Experiments on the effects of lead on growth of plaice (*Pleuronectes platessa*). Ann. Appl. Biol. 13: 168–176.

DORFMAN, D., AND W. R. WHITWORTH. 1969. Effects of fluctuations of lead, temperature, and dissolved oxygen on the growth of brook trout. J. Fish. Res. Bd. Canada 26: 2493–2501.

ELLIS, R. H. 1967. Effects of kraft mill effluent on the production and food relations of juvenile chinook salmon in laboratory streams. Nat. Counc. Air Stream Improv. Tech. Bull. 210: 1–55.

FUJIYA, M. J. 1961. Effects of kraft pulp mill wastes on fish. J. Water Pollut. Contr. Fed. 33: 968–977.

GRANT, B. F., AND P. M. MEHRLE. 1970. Chronic endrin poisoning in goldfish, *Carassius auratus*. J. Fish. Res. Bd. Canada 27: 2225–2232.

HERRMAN, R. B., C. E. WARREN, AND P. DOUDOROFF. 1962. Influence of oxygen concentration on growth of juvenile coho salmon. Trans. Amer. Fish. Soc. 91: 155–167.

HOWARD, T. E., AND C. C. WALDEN. 1965. Pollution and toxicity characteristics of kraft mill effluents. TAPPI 48: 136–141.

MS 1967. Haematocrit variation at sub-lethal concentrations of kraft mill wastes. Paper presented at 97th Annual Conference, Amer. Fish. Soc. 16 p.

1971. Effluent characteristics of bleached kraft pulp mills. Pulp Pap. Mag. Can. 72: 73–79.

PICKERING, Q. H. 1968. Some effects of dissolved oxygen concentrations upon the toxicity of zinc to the blue gill, *Lepomis macrochirus*. Raf. Water Res. 2: 187–194.

SCHAUMBERG, F. D., T. E. HOWARD, AND C. C. WALDEN. 1967. A method to evaluate the effects of water pollutants on fish respiration. Water Res. 1: 731–737.

SPRAGUE, J. B., AND D. W. MCLEESE. 1968a. Toxicity of bleached kraft mill effluent to larval and adult lobsters and Atlantic salmon. Pulp Pap. Mag. Tech. Pap. T431–T433.

1968b. Toxicity of kraft mill effluent to larval and adult lobsters and juvenile salmon. Water Res. 2: 753–760.

1968c. Different toxic mechanisms in kraft mill effluent for two aquatic animals. Water Res. 2: 761–765.

STEWART, N. E., D. L. SHUMWAY, AND P. DOUDOROFF. 1967. Influence of oxygen concentration on the growth of juvenile large mouth bass. J. Fish. Res. Bd. Canada 24: 475–494.

TOKAR, E. M., AND E. L. OWENS. 1968. Growth, food consumption and swimming ability of juvenile chinook salmon. Nat. Counc. Air Stream Improv. Tech. Bull. 217: 1–46.

WALDEN, C. C., AND T. E. HOWARD. MS 1971. The nature and magnitude of the effect of kraft mill effluents on salmon. Paper presented at Int. Symp. Ident. Meas. Environ. Pollut. Ottawa. 30 p.

WALDEN, C. C., T. E. HOWARD, AND G. C. FROUD. 1970. A quantitative assay of the minimum concentrations of kraft mill effluents which affect fish respiration. Water Res. 4: 61–68.

WALDEN, C. C., T. E. HOWARD, AND W. J. SHERIFF. 1971. The relation of kraft pulp mill operating and process parameters to pollution characteristics of the mill effluent. Pulp Pap. Mag. Can. 72: 81–87.

WARREN, C. E., AND G. E. DAVIS. 1967. Laboratory studies on the feeding, bioenergetics and growth of fish, p. 175–214. *In* S. D. Gerking [ed.] The biological basis for freshwater fish populations. Blackwell Scientific Publications, Oxford.

WERNER, A. E. 1963. Sulphur compounds in kraft mill effluents. Can. Pulp Pap. Ind. 16: 35–43.

Appendix I

Some deaths of fish occurred in 0 and 10% BKME from causes other than BKME exposure. Several fish

in 0% BKME died from salmon kidney disease, so the group was terminated after 14 days of the postexposure phase. This disease appeared not to affect growth, as is shown by comparing growth rate and conversion efficiency for this group with 1.0 and 2.5% BKME (Fig. 3A and 4). Condition factor and dry weight were also unaffected. The disease appeared to be present at a low chronic level in about one-third of this group of fish, suddenly becoming acute so that fish died before effects on growth could be detected, a feature which has been noted before. Following identification of the disease, all fish sampled for dry weight determinations were examined as were all fish at the end of the experiment. No further incidents were found.

Two-thirds of 10% BKME fish died on the 57th day of the BKME-exposure phase following a mechanical failure. All the fish were weighed and measured immediately, dead fish being used for dry weight determinations. The guts of the fish were still full at death; consequently, the mean weight tended to be slightly high, elevating growth rate and conversion efficiency. Condition factor was also elevated for the last measurement of the BKME-exposure phase (Fig. 2). Percentage dry weight was not affected by food retention, as the water content of food in the gut reaches the same percentage as that of the body within 15 min of feeding (Brett 1971). These fish were not sampled again for dry weight until the end of the experiment.

Distribution and Morphological Variation of *Lampsilis radiata* (Pelecypoda, Unionidae) in Some Central Canadian Lakes: A Multivariate Statistical Approach

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GREEN, R. H. 1972. Distribution and morphological variation of *Lampsilis radiata* (Pelecypoda, Unionidae) in some central Canadian lakes: a multivariate statistical approach. J. Fish. Res. Bd. Canada 29: 1565–1570.

Discriminant analysis performed on 62 samples containing *Anodonta grandis* and 52 samples containing *Lampsilis radiata* collected from 19 lakes in central Canada indicated separation in terms of an environmental factor which was interpreted as suitability of water chemistry for construction of shell. A canonical correlation analysis on morphological and environmental variables for 70 specimens of *L. radiata* yielded two highly significant canonical variates which related components of environmental variation to components of morphological variation. One was interpreted as variation in water turbulence, which primarily affected overall size. The other was similar to the factor which separated *A. grandis* from *L. radiata* and was interpreted similarly. Shell weight, or thickness, rather than overall size, was affected. Environments were successfully predicted for shells from two locations not included in the original analysis.

GREEN, R. H. 1972. Distribution and morphological variation of *Lampsilis radiata* (Pelecypoda, Unionidae) in some central Canadian lakes: a multivariate statistical approach. J. Fish. Res. Bd. Canada 29: 1565–1570.

L'analyse discriminante effectuée sur 62 échantillons contenant *Anodonta grandis* et 52 échantillons contenant *Lampsilis radiata*, recueillis dans 19 lacs du Canada central, démontre qu'il existe entre les deux espèces une distinction liée à un facteur du milieu, que nous avons identifié comme étant une composition chimique de l'eau favorable à la formation de la coquille. L'analyse de la corrélation canonique entre les variables morphologiques et de l'environnement de 70 spécimens de *L. radiata* donne deux variables aléatoires hautement significatives, reliant les facteurs du milieu et les éléments des variations morphologiques. Nous avons interprété une de ces variables comme étant une variation de la turbulence de l'eau, qui affecte surtout la taille globale. L'autre est semblable au facteur séparant *A. grandis* de *L. radiata*, et est interprétée de la même façon. Le poids de la coquille, ou son épaisseur, plutôt que la taille globale, est affecté. Il a été possible de prédire avec succès, en deux endroits non inclus dans l'analyse originelle, que ces milieux seraient favorables à la croissance des coquilles.

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THIS study was carried out to determine the distribution of *Lampsilis radiata* in lakes of central Canada in relation to environmental factors, and to determine the components of morphological variation in *L. radiata* which are related to identifiable components of environmental variation. Multivariate statistical methods have been applied in both aspects of the study.

Multivariate methods have been used to analyze either species distributions or morphological variation among geographical areas for wolves (Jolicoeur 1959), deer (Rees 1970), salmon (Calaprice 1971), chironomids (Rosenberg 1971), and bivalve molluscs (Green 1971). However, none of these authors was attempting to relate patterns of morphological variation to variation in environmental factors.

Environmental factors are known to influence morphology in bivalve molluscs. For example, Fairbridge (1953) has demonstrated that the growth rate of the Tasmanian commercial scallop *Notovola meridianalis* varies among localities, and Abbott and Jensen (1967) state that recent marine mollusc immigrants to Bermuda shallows grow larger than elsewhere because of environmental influences. In fresh water environmental factors can strongly influence shell morphology in sphaeriids (Herrington 1962) and in unionids (Parmalee 1967).

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Distribution of Lampsilis radiata

A total of 382 samples collected from 37 lakes in Saskatchewan, Manitoba, and northwestern Ontario contained 22 species of live bivalve molluscs. For a summary of locations sampled, sampling methods, and species collected, see Green (1971). *L. radiata* and *Anodonta grandis* were the only unionids collected in more than two samples, and were also the only unionids collected from more than one lake. The unionid fauna of this region is far more diverse in rivers and streams than in lakes. The other four unionid species (*Lasmigona complanata*, *Amblema costata*, *Lampsilis ovata ventricosa*, and *Ligumia recta*) were collected from habitats approaching the lotic: off river mouths in large lakes, in a channel with high current, and in a small artificial lake on a river. In the following consideration of the distribution of *L. radiata* among and within lakes of this region, it is therefore assumed that ecological factors related to separation from, or replacement by, *A. grandis* are of greatest interest.

In the analysis by Green (1971), the differences in ecological distribution between *L. radiata* and *A. grandis* were slight, but the large differences between unionids and sphaeriids, and among sphaeriid species, probably tended to obscure the smaller differences between these two ecologically more similar species. The geographic distributions of the two species obviously differ. In the large, high alkalinity lakes of the Lake Agassiz basin *L. radiata* predominates, although *A. grandis* is also present. In oligotrophic lakes of the Precambrian Shield and the Lake Agassiz escarpment *A. grandis* is the more common species.

Discriminant analysis was performed on 62 samples containing *A. grandis* and 52 samples containing *L. radiata* collected from 19 lakes in Manitoba and Ontario. In the analysis, the samples are initially distributed in a nine-dimensional space because measurements on nine environmental variables are associated with each sample. The rationale for the choice of these variables (Table 1), the statistical assumptions, the techniques of measurement, and the transformations used are discussed in Green (1971). Details of calculation are presented by Rao (1952) and Cooley and Lohnes (1962). The maximum number of discriminant functions is always equal to the number of variables (m), or the number of groups (g) less one, whichever is the smaller. Therefore this analysis can yield only one discriminant function ($g - 1 = 1$).

The separation of these two species in the defined environmental space is highly significant (χ^2 ($m(g-1) = 9$ df) $= 40.7$). That is, the probability of drawing, at random, two groups as spatially separate as these from the given nine-dimensional multivariate swarm is less than 0.01. Therefore I conclude that these two species differ in their distributions with respect to this set of environmental variables.

Table 1. Discriminant analysis on 62 samples containing *Anodonta grandis* and 52 samples containing *Lampsilis radiata*. The discriminant vector is standardized and normalized, and coefficients greater than $\pm$ 0.3 are italicised.

Environmental parameters	Discriminant vector
*p*H	*–0.567*
Alkalinity	*0.702*
Calcium	–0.037
Total hardness–calcium	–0.163
Depth	0.076
NaCl	*0.368*
Sediment mean particle size	0.011
Sediment sorting coefficient	0.122
Sediment % organic content	–0.029

The standardized and normalized vector of discriminant function coefficients is given in Table 1. It is obvious that the sediment variables have no bearing on the ecological separation of these two species. The important variables appear to be *p*H, alkalinity, and sodium chloride concentration. Alkalinity and *p*H are usually positively correlated, but here have coefficients of opposite sign, suggesting that some unmeasured variable which increases as alkalinity increases, but also increases as *p*H decreases, is involved. Such a variable is free CO_2, which, at a given *p*H, increases as alkalinity increases because the total CO_2-bicarbonate-carbonate pool increases. For a given alkalinity, free CO_2 increases as *p*H decreases because the equilibrium shifts toward free CO_2. Free CO_2 in the water is probably the main source of both the carbonate and the organic matrix portions of the shell (Wilbur 1964); therefore, this interpretation makes good physiological sense. Since primary producers utilize free CO_2 for photosynthesis, this discriminant function may also reflect variation in productivity; the lakes in which *A. grandis* is the characteristic unionid certainly tend to be more oligotrophic than lakes where *L. radiata* predominates. In the analysis of ecological factors separating 10 unionid and sphaeriid species in lakes of this region (Green 1971), it was found that discriminant functions accounting for 80% of the separation could be interpreted most easily as trophic factors, despite the obvious bias toward chemical and physical variables in the choice of parameters.

The discriminant vector (Table 1) was used as a vector of coefficients in a linear additive equation (-0.567 *p*H $+ 0.702$ alkalinity $- 0.037$ calcium..., etc.) to calculate a discriminant score for each of the original 114 samples. When plotted as in Fig. 1, it is apparent that *L. radiata* dominates at the low

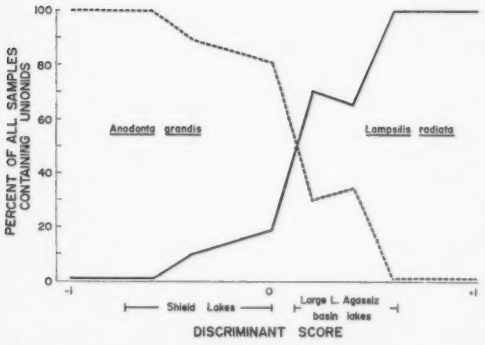

FIG. 1. The distribution of occurrence of *Anodonta grandis* and *Lampsilis radiata*, expressed as a percentage of all samples containing unionids, in relation to the discriminant scores. The ranges of discriminant scores for samples from shield lakes, and from large Lake Agassiz basin lakes, are shown.

*p*H, high alkalinity, and high NaCl end of the spectrum, while *A. grandis* dominates at the other end. Whether high NaCl concentration is actually limiting for *A. grandis*, or is simply a characteristic of lakes where *L. radiata* predominates for other reasons, is at this point uncertain. In any case, it seems a reasonable hypothesis that *L. radiata* is replaced by *A. grandis* for reasons that have something to do with the ability to construct new shell. The fact that the often paper-thin shell of *A. grandis* requires far less $CaCO_3$ for a given tissue weight of animal than does the heavy shell of *L. radiata* is consistent with this hypothesis. If such a factor limits the distribution of *L. radiata*, then it may be expected to also influence shell morphology where the species does occur.

In interpreting this analysis, the constraints involved should be kept in mind. One is the mathematical impossibility of generating more than one discriminant function for the separation of two groups. Another is that the demonstrated separation is based on the nine environmental variables that were used. One can show that two species are ecologically separated (are "in different niches") on the basis of a significant discriminant function of a given set of environmental variables, but one cannot demonstrate that they are not ecologically separated (are "in the same niche") on the basis of a lack of separation on a particular set of environmental variables. Similarly, one cannot know what additional factors acting to separate the species might have been revealed if additional environmental variables had been included. For example, if the mean size of food particles in the foregut had been used as a variable, it might have provided an additional, and clearly trophic, basis of separation. Significant separation in such analyses should be treated as a sufficient, but not necessarily complete, description of ecological separation.

VARIATION OF SHELL MORPHOLOGY OF L. RADIATA IN RELATION TO ENVIRONMENT

Eight quantitative morphological variables were determined for each of 70 specimens of *L. radiata* collected from nine lakes: (1) total length (anterior–posterior), (2) maximum height (dorsal–ventral), (3) maximum thickness (lateral), (4) distance from the anterior slope of the umbo to the posterior end of the shell, (5) weight of both valves, (6) distance from the pseudocardinal tooth to the posterior end of the lateral tooth in the right valve, (7) distance from the anterior margin of the anterior adductor muscle scar to the posterior margin of the posterior adductor muscle scar, and (8) inside volume of the shell.

All specimens had been preserved in 70% ethanol for approximately the same length of time. Specimens obviously of young age, as judged by number of annual rings, were eliminated from the analysis. All length measurements were made with vernier calipers, and inside volume of shell was assumed to be equivalent to the volume of water which both valves would hold. The values for all the above variables were logtransformed so that all allometric relationships would be linear.

A canonical correlation analysis (Hotelling 1936) was performed on these 70 specimens for the nine environmental variables used in the discriminant analysis above and the nine morphological variables (including the sex of the specimen, coded 0–1, since sexual dimophism of the shell is pronounced in this species).

Given two sets of variables (environmental and morphological in this case), a canonical correlation is the maximum correlation between linear functions of the two sets, with the linear functions chosen so as to maximize the correlation with the restriction that they be independent of previously derived linear combinations. Details of calculations are given by Cooley and Lohnes (1962).

The results of the canonical correlation analysis are shown in Table 2. Two canonical variates (CV) are significant at the 0.01 level, while the third CV extracted is not significant at the 0.05 level. The effect of CV I on shell morphology appears to be modification of overall length and proportions; valve weight is relatively unaffected. The empirical interpretation of CV I is: *L. radiata* occurring at greater depths in relatively high calcium and NaCl, and low magnesium, waters tend to have shells whose overall length, particularly in relation to length of hinge teeth and position of umbo, is reduced. Further interpretation of the ecological factor involved will be given later.

The empirical interpretation of CV II is: *L. radiata* occurring at greater depths in waters with relatively

TABLE 2. Canonical correlation analysis for 70 specimens of *Lampsilis radiata* on nine morphological parameters and nine environmental parameters. The vectors are standardized and normalized, and coefficients greater than ±0.3 are italicised.

Canonical variates	I	II	III
Canonical correlation	0.826	0.763	0.524
Degrees of freedom	81	64	49
Chi-square	176[a]	107[a]	54 ns
Predictor variables			
*p*H	–0.023	*–0.310*	
Alkalinity	–0.253	*0.747*	
Calcium	*0.509*	–0.278	
Total hardness–calcium	*–0.432*	–0.068	
Depth	*0.538*	*0.308*	
NaCl	*0.393*	*–0.389*	
Sediment mean particle size	0.018	0.010	
Sediment sorting coefficient	–0.197	–0.089	
Sediment % organic content	–0.077	0.097	
Criterion Variables			
Length	*–0.584*	0.270	
Height	–0.142	–0.064	
Thickness	–0.231	–0.167	
Umbo–posterior end	*0.315*	–0.081	
Valve weight	0.152	*0.759*	
Tooth length	*0.467*	*–0.431*	
Scar length	*–0.456*	*–0.332*	
Volume	0.184	0.125	
Sex	–0.053	–0.027	

[a]Significant at 0.01 level.

high alkalinity, low *p*H, and low NaCl concentration tend to have heavier shells with reduced length of hinge teeth and interscar distance. The environmental vector is similar to the discriminant vector for the separation of *L. radiata* and *A. grandis* distributions. Depth is not involved in the latter, and the coefficients for NaCl concentration have opposite sign. The latter may be explained by the fact that *L. radiata* in general tend to occur in lakes with higher NaCl levels than do *A. grandis*. As for depth, the two species occur at about the same depth on the average, but the depth at which *L. radiata* occur is far more variable from lake to lake than is the depth at which *A. grandis* occur.

Apparently sediment type is as irrelevant in determining shell morphology of *L. radiata* as it is in separating *L. radiata* from *A. grandis*. Shell height, thickness, and inside volume appear to be conservative variables in relation to environmental variability.

For each of the 70 specimens of *L. radiata*, the morphological CV I and CV II scores were calculated from the morphological data. The position of each specimen was then plotted in the two-dimensional space defined by CV I and CV II, and specimens collected from the same lake, or the same area in a large lake, were circumscribed by a closed curve. Lakes or areas of large lakes represented by only one sample were eliminated. The result is shown in Fig. 2.

High values of CV I are from protected, narrow lakes with no wave action and steady water flow, such as the artificial lakes, Minnedosa and St. Malo. The lowest values are from open, wave-swept shorelines of Lake Winnipeg. The positions of the other lakes and lake areas in relation to CV I exactly follow the sequence that would be expected if CV I represents water turbulence. The environmental vector of coefficients (Table 2) is consistent with this interpretation. The specimens from the most turbulent habitats occur at the greatest depths, often in waters with the highest Ca/Mg ratio and above-average NaCl concentrations.

To provide a more objective test of this hypothesis, the maximum fetch was determined from topographic sheets for each of the locations sampled (Fig. 2).

Maximum fetch for areas sampled in miles was:

St. Malo	<1
Minnedosa	1
First Cranberry	5
Winnipeg, Berens R. mouth	9
Winnipeg, Hecla channel	10
St. Martin	16
Manitoba, at outlet	23
Winnipegosis, west shore	24
Winnipeg, Willow Pt.	45
Manitoba, Delta	47
Winnipeg, Gimli	50
Winnipeg, Dauphin R. mouth	89

These values do not take into account such factors as orientation of shoreline in relation to prevailing winds, height of shoreline, or depth of water offshore from the area sampled. However, they should provide some indication of degree of exposure to wind and wave action. Agreement with the sequence in Fig. 2 is good. The contrast between a shell from a turbulent environment in Lake Winnipeg off the Dauphin River mouth and a shell from a protected environment in Minnedosa Lake (both of which have approximately the same CV II scores) is shown in Fig. 3A and 3B. The turbulent environment produces reduced overall size, but similar shell thickness in proportion to overall size.

Lakes and areas of large lakes with similar water chemistry fall on similar values of CV II (Fig. 2). The three areas from the south basin of Lake Winnipeg (Hecla Island channel, and off-shore from

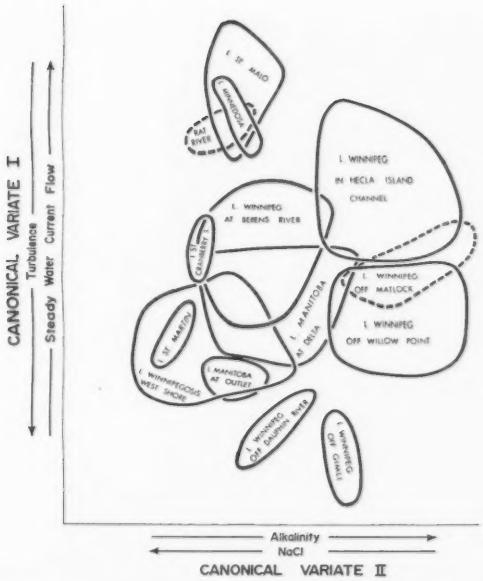

FIG. 2. The distribution of lakes and lake areas by morphological type of *L. radiata* shell. Each closed curve contains all samples from the given lake or lake area, as defined by CV I and CV II scores (solid curves = locations used in the analysis from which the canonical variates were derived, dashed curves = locations not used in the original analysis).

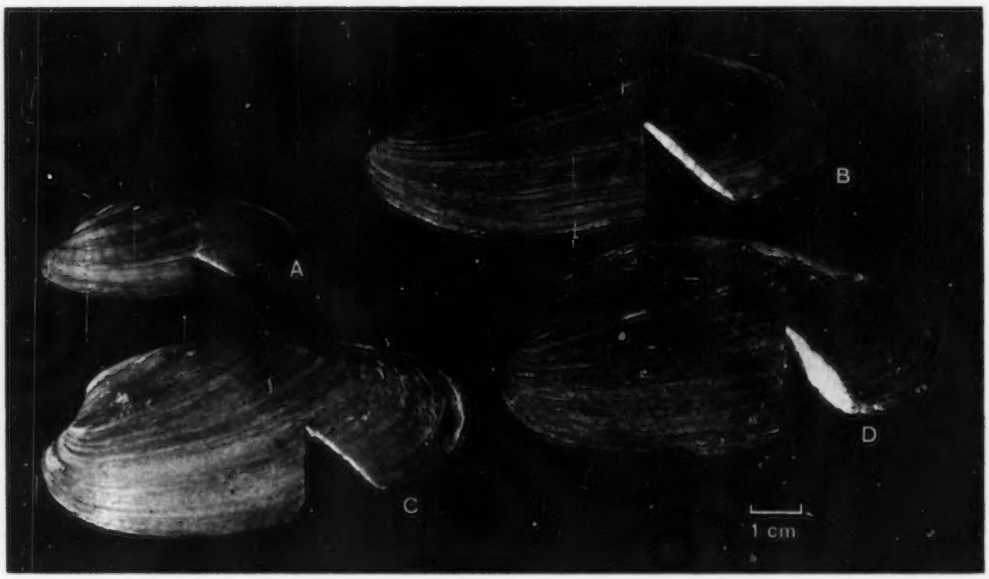

FIG. 3. Four *Lampsilis radiata* shells illustrating the effects of two different environmental factors on shell morphology: turbulence, and suitability of the water chemistry for $CaCO_3$ shell deposition. A, Lake Winnipeg off-shore from the Dauphin River; B, Minnedosa Lake; C, Lake St. Martin; D, Lake Winnipeg off-shore from Willow Point. See text for discussion.

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Willow Point and Gimli) have the highest values. Waters of high alkalinity, but also of high *p*H and NaCl content, such as Lake St. Martin and Lake Winnipegosis, have the lowest values. Intermediate is a heterogeneous group, consisting of relatively soft waters (First Cranberry Lake and Lake Winnipeg at the mouth of the Berens River) and waters similar to Lakes Winnipegosis and St. Martin, but with lower NaCl concentrations (Lake Winnipeg at the Dauphin River mouth and Lake Manitoba). The most reasonable interpretation of CV II (and of the discriminant function separating *L. radiata* and *A. grandis*) is that it represents the suitability of the water chemistry for all of the physiological processes involved in deposition of the $CaCO_3$ shell.

By far the highest coefficient value in the CV II morphology vector is valve weight (Table 2), and the values of the *p*H and alkalinity coefficients suggest the role of the CO_2–bicarbonate pool. The relation with valve weight, rather than overall size, suggests that suitability of the water chemistry for shell deposition, rather than in situ primary productivity, is the more important factor here. It also appears that high NaCl concentration has a deleterious effect on shell deposition. Given the value and sign of the discriminant vector coefficient in Table 1, *A. grandis* is presumably even more sensitive to high NaCl than is *L. radiata*. The sensitivity of unionids to NaCl concentration is in agreement with Cvancara (1967), who lists "chloride content" as a significant limiting factor affecting the distribution of unionids in the Red River valley, North Dakota. The contrast between shell thickness of *L. radiata* from a high NaCl, high *p*H-relative-to-alkalinity environment in Lake St. Martin, and from a low NaCl, high alkalinity-relative-to-*p*H environment in Lake Winnipeg off-shore from Willow Point (both of which have approximately the same CV I scores) is shown in Fig. 3C and 3D. Similar overall size, but reduced shell thickness, is characteristic of the first environment in comparison to the second.

Prediction of Environment From Lampsilis radiata Morphology

The canonical correlation analysis has produced a model which separates *L. radiata* shell morphologies on two statistically significant combinations of environmental variables (Table 2), describing reasonably well-separated environmental types (Fig. 2). However, it remains to be shown that the model is predictive as well as descriptive; that is, can the shell morphology of *L. radiata* from locations not included in the original analysis which generated the model be used to predict the environments in which they live? Two tests of the predictability of the model were made.

Valves from *L. radiata* collected offshore from Matlock, in Lake Winnipeg, were used in the first test. Five paired valves (two males and three females) were used to calculate CV I and CV II scores from the morphological parameters in the same manner as previously. Matlock, Manitoba, is at the southwest corner of the south basin of Lake Winnipeg. The predicted environment should therefore have CV II values similar to those for the Lake Winnipeg south basin specimens included in the original analysis. Although the nearshore water off Matlock is exposed to the open south basin, the offshore depths are less at the south end of the lake than, for example, off Willow Point. Also, the Matlock area is less exposed to winds and wave action because of the orientation of the shoreline. Therefore CV I values should indicate an environment less turbulent than off Willow Point, but more turbulent than the Hecla Island channel. The environment predicted from the Matlock shells is shown as a dashed closed curve in Fig. 2, and agrees well with the prediction.

Three *L. radiata* (one male and two females) from approximately 1 mile below St. Malo Lake on the Rat River, Manitoba, were also used to calculate CV I and CV II scores. The CV II scores should be similar to those for St. Malo Lake. The St. Malo Lake CV I scores were characteristic of protected waters in which the highest wind velocities produce only surface ripples. In the river, wind effects are also virtually absent; however, when in spring flood the Rat River is quite turbulent, especially along the shoreline where *L. radiata* are most abundant. Therefore, CV I scores for the Rat River should, if different, be slightly lower than for St. Malo Lake. The predicted environment for Rat River *L. radiata* is also shown as a dashed closed curve in Fig. 2, and again agrees well with the prediction.

Potential Applications

This approach can be used to assess the nature and extent of environment-related morphological variation in a given species. Each independent environmental factor can be identified, tested for statistical significance, and assigned its relative importance. Geologists, paleoecologists, and archeologists often wish to describe the environment in which a particular stratum was laid down, and mollusc shells are common fossils in strata laid down in both salt and fresh shallowwater environments. Paleoenvironments could be described with respect to such characteristics as exposed shore vs. protected shore or saline vs. nonsaline.

Two precautions should be mentioned. The CV vectors and scores are expressed here in standardized

and normalized form, based on the means and variances of this particular set of variables determined for this particular region and range of habitat types. Use of these vectors for calculating scores from data collected elsewhere and standardized in relation to other means and variances would certainly yield misleading results. That is, all data must be standardized with respect to the same means and variances.

In any event, it would be dangerous to apply vectors calculated for one geographic region to data from another. Morphological clines are known for many species, particularly on latitudinal gradients; for example, Cvancara (1963) has demonstrated latitudinal clines in shell shape for three species of *Lampsilis*. Application to data from a different region would be particularly risky where there was a substantial latitudinal difference.

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ABBOTT, R. T., AND R. JENSEN. 1967. Molluscan faunal changes around Bermuda. Science 155: 687–688.

CALAPRICE, J. R. 1971. X-ray spectrometric and multivariate analysis of sockeye salmon (*Oncorhynchus nerka*) from different geographic regions. J. Fish. Res. Bd. Canada 28: 369–377.

COOLEY, W. W., AND P. R. LOHNES. 1962. Multivariate procedures for the behavioral sciences. John Wiley & Sons, Inc., New York, N.Y. 211 p.

CVANCARA, A. M. 1963. Clines in three species of *Lampsilis* (Pelecypoda: Unionidae). Malacalogia 1: 215–225.

1967. Mussels in the Red River Valley in North Dakota and Minnesota and their use in deciphering drainage history, p. 187–196. *In* W. J. Mayer-Oakes [ed.] Life, land and water (Proc. 1966 Conf. Environ. Stud. Glacial Lake Agassiz Reg.). Univ. Manitoba Press, Winnipeg, Man.

FAIRBRIDGE, W. S. 1953. A population study of the Tasmanian "commercial" scallop, *Notovola meridianalis* (Lamellibranchiata, Pectinidae). Aust. J. Mar. Freshwater Res. 4: 1–41.

GREEN, R. H. 1971. A multivariate statistical approach to the Hutchinsonian niche: bivalve molluscs of central Canada. Ecology 52: 543–556.

HERRINGTON, H. B. 1962. A revision of the Sphaeriidae of North America (Mollusca: Pelecypoda). Misc. Publ. Mus. Zool. Univ. Mich. 118: 74 p.

HOTELLING, H. 1936. Relations between two sets of variates. Biometrika 28: 321–377.

JOLICOEUR, P. 1959. Multivariate geographic variation in the wolf *Canis lupus* L. Evolution 8: 283–299.

PARMALEE, P. W. 1967. The fresh-water mussels of Illinois. Ill. State Mus. Pop. Sci. Ser. 8: 108 p.

RAO, C. R. 1952. Advanced statistical methods in biometrical research. John Wiley & Sons, Inc., New York, N.Y. 390 p.

REES, J. W. 1970. A multivariate morphometric analysis of divergence in skull morphology among geographically contiguous and isolated groups of white-tailed deer (*Odocoileus virginianus*) in Michigan. Evolution 24: 220–229.

ROSENBERG, T. 1971. Geographical variation of certain taxonomic characters in natural populations of *Tanytarsus heusdensis* (Diptera : Chironomidae) in Finland. Can. Entomol. 103: 432–444.

WILBUR, K. M. 1964. Shell formation and regeneration, p. 243–282. *In* K. M. Wilbur and C. M. Yonge [ed.] Physiology of mollusca. Academic Press, Inc., New York, N.Y.

Developmental Stages of *Caligus clemensi* (Copepoda: Caligidae)

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KABATA, Z. 1972. Developmental stages of *Caligus clemensi* (Copepoda: Caligidae). J. Fish. Res. Bd. Canada 29: 1571–1593.

Morphology of developmental stages of *Caligus clemensi* (Copepoda: Caligoida), a parasite of important commercial fish species, is described. The development cycle consists of two nauplius stages, one copepodid, four chalimus stages, and one preadult. This cycle is compared with ontogeny of five other species of Caligidae. The author concludes that one preadult stage has been missed in the cycle of *C. clemensi* and that evidence so far available points to existence of a ten-stage cycle of development, including two preadult stages and the adult.

KABATA, Z. 1972. Developmental stages of *Caligus clemensi* (Copepoda: Caligidae). J. Fish. Res. Bd. Canada 29: 1571–1593.

L'auteur décrit les stades de développement de *Caligus clemensi* (Copepoda: Caligoida), parasite de poissons de commerce importants. Le cycle comprend deux stades nauplius, un copépodite, quatre chalimus, et un préadulte. L'auteur compare l'ontogénie de cette espèce avec celle de cinq autres espèces de Caligidae. Il conclut qu'un stade préadulte a été omis du cycle de *C. clemensi* et que, selon nos connaissances actuelles, cette espèce possède dix stades de développement, y compris deux préadultes et un adulte.

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IN an earlier publication, Parker and Margolis (1964) referred to damage inflicted on fins of fingerling pink salmon, *Oncorhynchus gorbuscha*, by attached early stages of *Caligus clemensi*, a caligid copepod described in the same paper. In a number of reported instances, feeding activity of the larval copepod resulted in destruction of entire fins. Similar damage was mentioned again later (Kabata 1970). It is also known (Parker and Margolis 1964) that *C. clemensi* parasitizes other economically important fishes. *Salmo gairdneri*, *Oncorhynchus keta*, and *Clupea pallasii* were listed among its hosts.

Our knowledge of ontogeny of caligid copepods is less than sufficient. Morphology of the chalimus stages varies from species to species considerably less than it does at the adult stage. Consequently, even investigators with reasonable knowledge of parasitic copepods are in some difficulty when confronted with the necessity of identifying larval caligids. Since *C. clemensi* is an important pest of commercially valuable species of Canadian Pacific fishes, it was judged relevant to the pursuits of fisheries research that its developmental stages should be described and its identification at all ages facilitated. Adults of the species can be identified by reference to the work of Parker and Margolis (1964).

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Another reason for writing this paper is an apparent lack of uniformity in the ontogeny of caligids studied so far. Various authors reported various numbers of stages in ontogeny of the caligid species they studied. It is difficult to accept that closely related species should differ extensively in the manner of their development. It was hoped that comparison of developmental stages of *C. clemensi* with those of previously studied species might clarify to some extent the nature of their ontogeny.

The stages described in this paper were obtained by Dr R. R. Parker, Pacific Biological Station, Nanaimo, B.C., by rearing *C. clemensi* from eggs. Fingerling pink salmon were exposed to infection in small beakers of sea water containing the copepodid stage of the parasite. Fish were examined at intervals and successive stages of ontogeny were removed from them and preserved in individual vials. In this way a complete series of stages was collected, representing the attached phase of ontogeny. Difficulties were encountered in securing stages following their separation from the frontal filament. Hence, as regards the preadult phase of development, no claim can be laid to absolute certainty of observations presented in this paper.

The specimens were examined, both entire and dissected, in Berlese's fluid under phase contrast and Nomarsky's interference phase illumination,

magnified up to ×900. Drawings were made with the aid of an eyepiece graticule and are all freehand.

First Nauplius

The body is irregularly oval (Fig. 1) with truncated anterior and posterior margins. Three pairs of appendages protrude laterally from the anterior half of the ventral surface. The posterior end is armed with two filiform balancers, diverging laterally. The length (10 specimens) ranges from 0.43 to 0.50 (average 0.46) mm.

The first antenna (Fig. 3–5) is uniramous, indistinctly divided into two segments of about equal length. The proximal segment is cylindrical, equipped with two unarmed setae arising from its ventral side. The distal segment is also cylindrical, with rounded tip (Fig. 4 and 5). The armature of the tip consists of apical and subapical structures. The former comprise a short aesthete (Fig. 4 and 5, ae) and two setae about twice as long as the aesthete, each armed with two strips of serrated membrane (omitted in drawings). The subapical structures comprise four (sometimes three) spines arranged along a spiral line, beginning on the dorsal surface near the tip and descending in a clockwise direction. The size of the spines diminishes from the tip towards the base. They are normally arranged in a series of three (or two), with the gap between them and the remaining spine (Fig. 5).

The second antenna (Fig. 6) has an unsegmented sympod apparently fused with the basal segments of the rami, so as to appear distally bifid. The endopod (ed) is shorter than the exopod and two-segmented. The basal segment is short and unarmed. The distal segment is longer and has a rounded tip armed with one subapical and three apical structures. The subapical is an unarmed seta arising from the dorsal surface of the segment. Apical structures comprise two long setae armed with a strip of serrated membrane and a row of pinnules (Fig. 8). A spiniform, bluntly tipped outgrowth is present at the base of the apical setae. The exopod (e) is apparently five-segmented. Its basal segment is unarmed (its proximal border is not discernible). The second segment is about as large as the first. On its inner side (facing the endopod) it has a short outgrowth that provides the base for a large seta. Segments three to five diminish in size. The setae of segments three and four resemble that of segment two in size and attachment. Segment five is barrel shaped, with a sharp dentiform projection on the outer side. Its seta is born on the apex and is of about the same diameter as the segment itself. All setae are armed in the same way as those of the endopod.

The mandible (Fig. 7 and 8) is biramous. Its sympod is unsegmented and indistinctly delimited from the basal segments of the rami. The endopod (ed) is two-segmented, with indistinct border between the segments (the basal segment is short, unarmed, and not visible in Fig. 7 because of the position of the mandible). The apex of the distal segment bears two setae armed as in Fig. 8. A short outgrowth (possibly an aesthete) with blunt, inflated tip, extending distally just beyond the tip of the ramus, arises from its outer margin. The exopod is four-segmented, more than half of its length being provided by the basal segment. Segments two and three are short and diminish in diameter. The terminal segment is barely broader than the seta it carries. All four segments carry a single seta each.

Second Nauplius

In its general appearance (Fig. 2), the second nauplius is barely distinguishable from the first. It is longer and its balancers are relatively smaller, but otherwise the two stages are very like each other. The only certain way to tell them apart is by finding within them the outlines of the following stage, which in the second nauplius is an unmistakable copepodid. The length of the second nauplius (10 specimens) varies from 0.49 to 0.58 (average 0.53) mm.

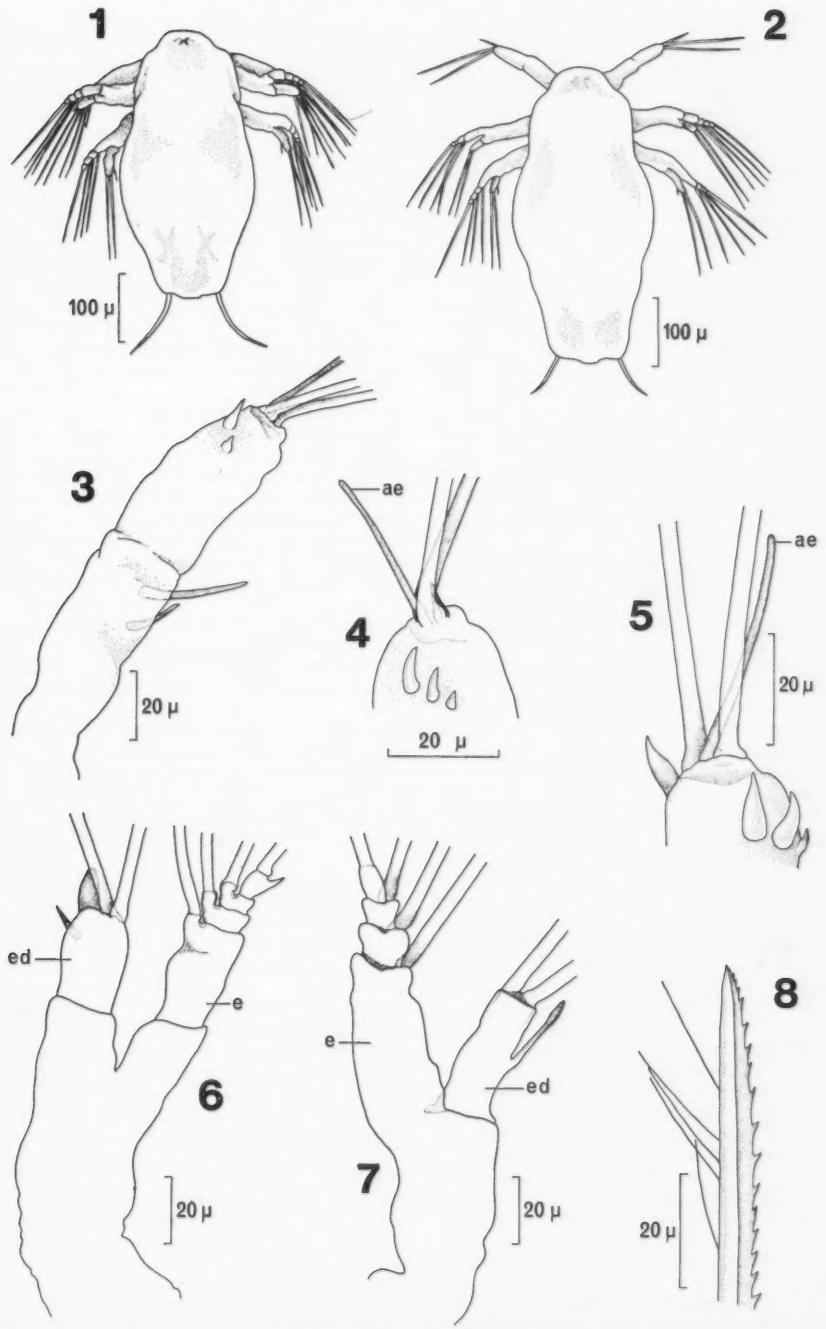
The appendages of the second nauplius are identical with those of the first.

Copepodid

The bodies of copepodids consist of two tagmata (Fig. 9 and 10). The anterior tagma, the cephalothorax, is formed by a fusion of the cephalic with the first two thoracic segments (those bearing maxillipeds and the first pair of swimming legs). Dorsally, this tagma is covered by a flat obovate shield. Posteriorly, it is transversely truncated. In addition to segmental appendages, the ventral surface of the cephalothorax carries also the rostral apparatus near the anterior margin, between the bases of the first antennae. Regrettably, in specimens available for examination the rostral area was in a condition which precluded detailed study. (By analogy with copepodids of *Caligus* previously studied (Izawa 1969) a rostrum can be expected to arise from that area and point in a posterior direction.) The dorsal shield carries a number of very fine, probably sensory setules. Two are present on the anterior margin (one on each side of the mid-dorsal line), a pair on each lateral margin at the level of the maxilliped bases, a pair near the centre in the region of the eyes, and a pair on either side near the posterolateral corner (Fig. 9).

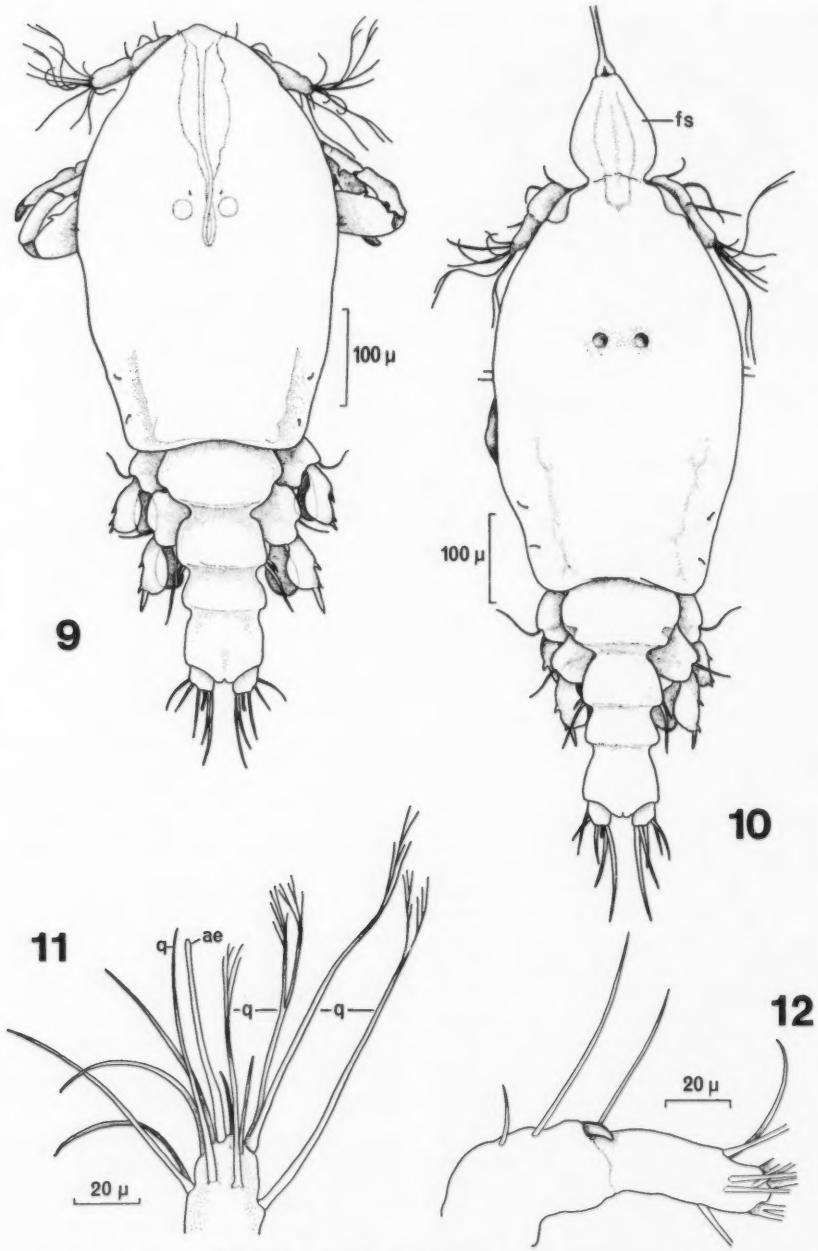
With extrusion of the frontal filament in the process of attachment a large flat structure is also everted. It is the frontal sac (Fig. 10, fs), destined to give rise to the frontal plates of the adult *Caligus*.

The posterior tagma consists of four segments, fairly clearly delimited from one another. The first of them is the third thoracic segment, bearing the second pair of swimming legs. It is short and about half as wide as the cephalothorax at the level of the maxilliped bases. The fourth thoracic segment is narrower than the third and carries in its posterolateral corners the rudiments of the third pair of legs. The fifth segment is smaller than its predecessor and carries no appendages. It is followed by a segment which represents a fusion of the abdomen and the still undifferentiated genital complex of the thorax. Heegaard (1947) aptly refers to it as pseudabdomen. It carries on its posterior margin a well-equipped caudal furca whose rami are mounted on either side of the anal slit. The length of copepodids



FIGURES 1–8. *Caligus clemensi*, nauplius stages.

FIG. 1. First nauplius, dorsal; FIG. 2. Second nauplius, dorsal; FIG. 3. First and second nauplius, first antenna, dorsal; FIG. 4. Same, tip, anterodorsal; FIG. 5. Same, tip, posterodorsal; FIG. 6. Second antenna, ventral; FIG. 7. Mandible, ventral; FIG. 8. Tip of mandibular seta, ventral. (ae, aesthete; e, exopod; ed, endopod).



FIGURES 9–12. *Caligus clemensi*, copepodid.

FIG. 9. Free swimming copepodid, dorsal; FIG. 10. Attached copepodid, dorsal; FIG. 11. Tip of first antenna, ventral; FIG. 12. First antenna, without apical armature, ventral. (ae, aesthete; fs, frontal sac; q, posterior apical setae).

(4 specimens) varies from 0.64 to 0.70 (average 0.66) mm.

The first antenna (Fig. 11 and 12) is a simple, cylindrical appendage bending at right angles near its base and indistinctly divided into two segments. The basal segment (Fig. 12) is armed with three unarmed setae; one short, and two very long. It also has a short but strong claw on its anterior margin, near the junction of the two segments. The terminal segment is cylindrical, its unevenly rounded tip carrying the apical armature (Fig. 11). The latter consists of 12 setae. Five of them are mounted on the posteroventral side of the tip. These setae (Fig. 11 q) are usually irregularly branched at their tips. Two of them arise out of a common base. The apex itself is occupied by an aesthete (Fig. 11, ae). In addition, long slender setae are found on the anterior side of the tip and two short ones between the aesthete and the bases of the two fused setae.

The second antenna (Fig. 13) is three-segmented. Its basal segment is short and armed with a backwardly projecting spiniform process. The main part of the appendage is formed by the middle segment, broader at its base and tapering distally. The third segment forms a terminal claw. It has a slightly inflated base, bends sharply, and tapers to a fine long tip. It is armed with a long, slender setiform process arising from near its base and with a small tubercle on its concave margin near the point of flexure.

The postantennary process (Fig. 15) is located near the lateral margin of the cephalothorax, not far from the claw of the second antenna. It is a short, dentiform outgrowth with bluntly rounded tip and uneven medial margin.

The mouth cone is essentially similar at all stages and will be described with the preadult. The mandible (Fig. 14) is about 70 μ long and appears to consist of three ill-defined parts. The basal part is inflated and conical, the middle part narrow and cylindrical, and the distal part flat, blade-like, and armed with a series of ten fairly uniform teeth.

The first maxilla (Fig. 16) consists of an elongate conical process, soft and easily disarranged by handling (r), and a papilla (a) surmounted by one long and two shorter setae. It is located on the posterolateral side of the mouth cone and is posterior to the base of the mandible.

The second maxilla (Fig. 17 and 18) consists of three parts. Its junction with the surface of the cephalothorax gives an impression of a short, obsolete segment. A short seta at this point has been reported in other species (Izawa 1969) and seen in later stages of *C. clemensi* (Fig. 30). Though unobserved, it might also be present in this copepodid. The basal part constitutes little less than a half of the length of the second maxilla. It articulates with the second part by an elbow-like joint (Fig. 17). It is the most robust part of the appendage and is unarmed. The second part is subcylindrical and slightly longer than the basal. It bears on its anterior margin a long strip of serrated membrane. Another strip of membrane, not serrated, extends along the posterior margin. A rounded, flat lappet with margins beset with short, bristling setae is present in the posterodistal corner. Distally, the second part is indistinctly divided from the third. The latter is short, slightly curving, and attenuated into a sharp tip. Its ventral surface is densely covered with irregularly scattered short setae (Fig. 17). From that aspect can be seen also what appears like a row of short spiniform setae on its anterior margin. Seen from the dorsal side, however (Fig. 18), this row can be seen to be composed of many rows of spinules, all beginning on the anterior margin and running along it for a short distance before turning transversely across the dorsal surface. Up to 10 rows are present, though the number appears to vary from one specimen to another.

The maxilliped (Fig. 19) consists of two parts, but its segmentation is not clear. The main part, or corpus maxillipedis, is slightly flattened, long, and narrow. Its medial margin is armed with three spiniform processes; one at the base, one near the second part, and the third about half way between the two. The second part, or subchela, consists of three ill-delimited segments. The basal segment is cylindrical and unarmed, the middle one is short and armed with a long spiniform process on its inner margin, and the distal is long and slender, curving at the tip, and armed with another spiniform process. The length of the subchela exceeds that of the corpus.

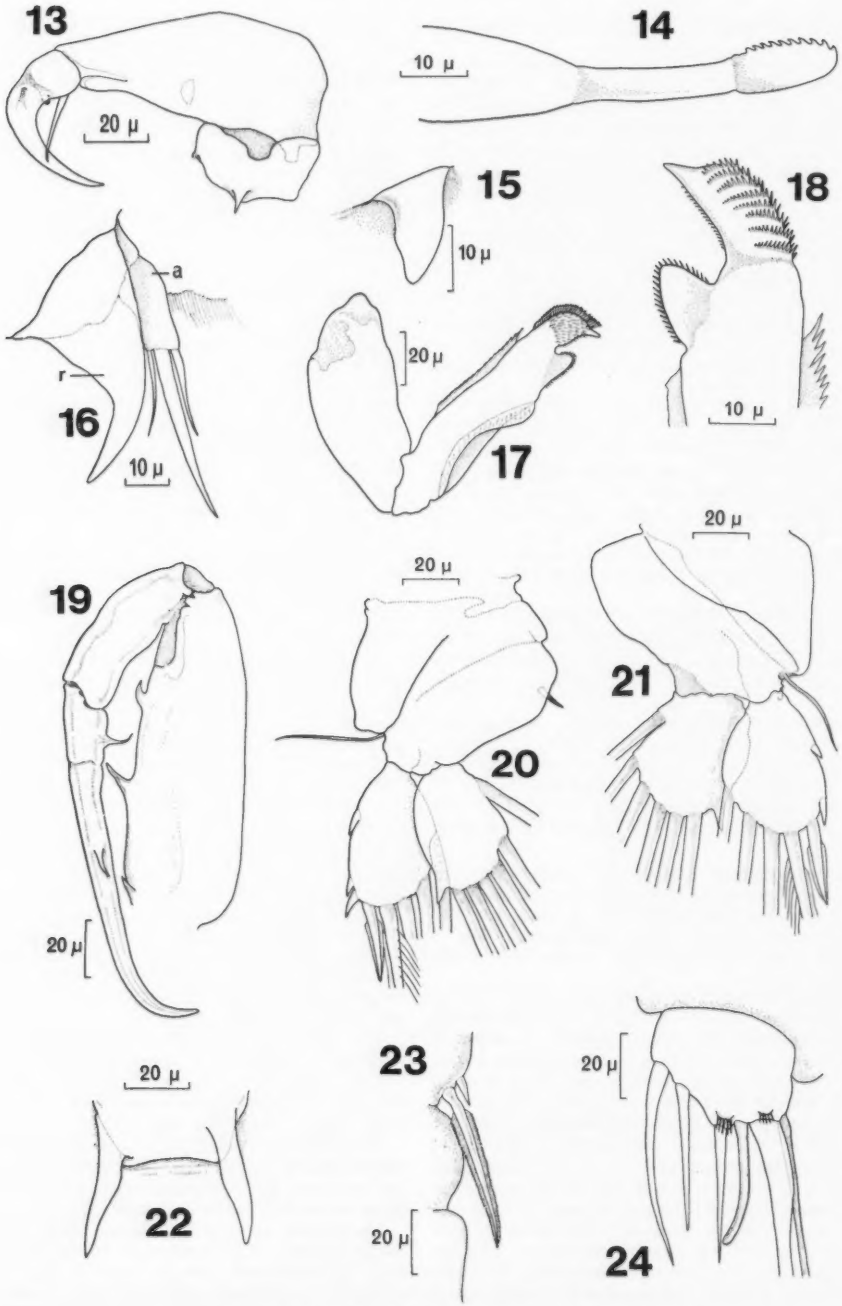
The sternal furca (Fig. 22) is in the midventral line behind the bases of the maxillipeds and in front of the first swimming legs. It consists of two styliform, fairly soft tines. The box of the furca (Parker et al. 1968) is not apparent.

The first swimming leg (Fig. 20) is biramous and linked with its opposite number by means of an interpodal bar. Its sympod is indistinctly two-segmented. The distal segment carries on its lateral margin, near the base of the exopod, a long, slender seta. Another, shorter seta is found on the medial margin, some distance from the base of the endopod. The rami are both one-segmented. The lateral margin of the exopod is armed with three dentiform processes. Its distal, and a part of its lateral, margin are occupied by five setae. The lateral seta is short and has strips of membrane along two margins. The second is much longer and has a similar membrane along the lateral, and pinnules along the medial, margin. The remaining three setae are long and pinnate. The endopod has a spiniform process (not delimited from the segment) in the posterolateral corner. It has one pinnate seta on its medial margin and five similar setae on its distal margin.

The second leg (Fig. 21) closely resembles the first, with few differences in armature. There is no seta on the medial margin of the sympod. The exopod has only two dentiform processes on its lateral margin. The endopod is the same as in the first leg.

The rudiment of the third leg (Fig. 23) is in the form of two setae of unequal length. One is very short, pointed, and unarmed, the other much longer and provided with strips of serrated membrane on two margins.

The caudal ramus (Fig. 24) has medial and lateral margins parallel to each other. Its lateral side is shorter than its medial, so that its posterior margin slopes obliquely in its lateral part. It is armed with six setae. Two long pinnate setae (cut off at $\frac{1}{3}$ of their length in Fig. 24) are present near the posteromedial corner, one



FIGURES 13–24. *Caligus clemensi*, copepodid.

FIG. 13. Second antenna, ventral; FIG. 14. Mandible, lateral; FIG. 15. Postantennary process, ventral; FIG. 16. First maxilla, ventral; FIG. 17. Second maxilla, entire, ventral; FIG. 18. Same, tip, dorsal; FIG. 19. Maxilliped, ventral; FIG. 20. First leg, ventral; FIG. 21. Second leg, dorsal; FIG. 22. Sternal furca, ventral; FIG. 23. Third leg, dorsal; FIG. 24. Caudal ramus, ventral (pinnules omitted). (a, papilla of first maxilla; r, process of first maxilla).

slender, the other with inflated base. Close to the latter is an aesthete-like seta, unarmed and with a rounded tip. There are three more short pinnate setae (pinnules omitted in Fig. 24) on the sloping part of the posterior margin. Close to the base of the presumed aesthete, on the ventral surface of the ramus, there are two comb-like structures, each consisting of a fold of cuticle and a row of short, stiff setules.

First Chalimus

The most characteristic feature of this stage (Fig. 25) is the absence of distinct segmentation. Boundaries between the anterior and posterior tagma, as well as between the individual segments of the latter, are marked by shallow grooves. Judging from the position of these grooves and from the proportions of the body, metamorphosis from the copepodid into the first chalimus stage has not been accompanied by appearance of additional segments. The base of the frontal sac is now much wider, so that this structure forms an anterior, subtriangular extension of the cephalothorax. The base of the frontal filament (Fig. 26) shows signs of subdivision, both longitudinal and transverse. The latter, however, is not very clear and cannot be accepted as evidence of additional material having been added to the base of the filament in the course of the moult. The armature of the appendages has been generally reduced in length. Length of chalimus (10 specimens) varies from 0.84 to 1.00 (average 0.91) mm.

The first antenna (Fig. 27) is largely similar to that of the preceding stage, except for reduction in the length of its setae and disappearance of the claw from the distal end of its first segment.

The second antenna (Fig. 28) now appears to consist of two parts only, with poorly defined articulation between them. The basal part is squat, broad, and unarmed. The terminal part bears a vague resemblance to the subchela of a maxilliped. It has a broad base and a tip set off from the rest of the appendage by a constriction. The tip is armed with two setae of unequal length.

There is no postantennary process.

The mandible (Fig. 29) has assumed its definitive structure by intercalation of a short segment between the base and the shaft. Its dentiferous margin has now 12 teeth.

The first maxilla remains unchanged from the preceding stage, though it has become relatively smaller.

The armature of the second maxilla (Fig. 30) is reduced by comparison with the copepodid stage. A short seta arises from the surface of the cephalothorax near the base of this appendage. There are no membranes along the margins of the second part. The subterminal lappet has a row of well-spaced, sturdy denticles along its margin. They are partly obscured by fine bristles, arranged in a row on the dorsal and ventral surfaces of the lappet. Similar bristles are present on the distal part, as its sole armature. The tip terminates in a fine, sharp point.

The maxilliped (Fig. 31) has lost three spiniform processes from its medial margin. Its subchela is now shorter than its corpus and no longer distinctly divided into three segments. It ends in a slightly curving, slender claw, with a small denticle near its tip and a seta at the base.

A sternal furca is absent.

The first swimming leg (Fig. 32) has retained its structure but is diminished in size and shows changes in armature. The most peculiar feature, not encountered in later stages, is the papilliform outgrowth on the medial margin of the sympod, near the base. On the other hand, there is no interpodal bar. The sympod is no longer clearly divided into two segments but it is equipped with a lateral seta at the base of the exopod. The latter is relatively more elongate. Its armature has retained its eight elements, though much modified. There are five unarmed setae on the distal margin. Short, dentiform outgrowths are present on the lateral (one) and medial (two) margins. The only remaining armature of the endopod is a single seta on its distal margin. This seta is unarmed and unusually long (only a half is shown in Fig. 32) so that it crosses with the corresponding seta of the opposite leg.

The second leg (Fig. 33) shows similar changes. Its exopod is armed with seven setae; three longer ones on the distal margin, and two shorter ones on both medial and lateral margins. The endopod has two long setae on the distal margin and one very short one on the medial margin. There are also two dentiform processes on that margin.

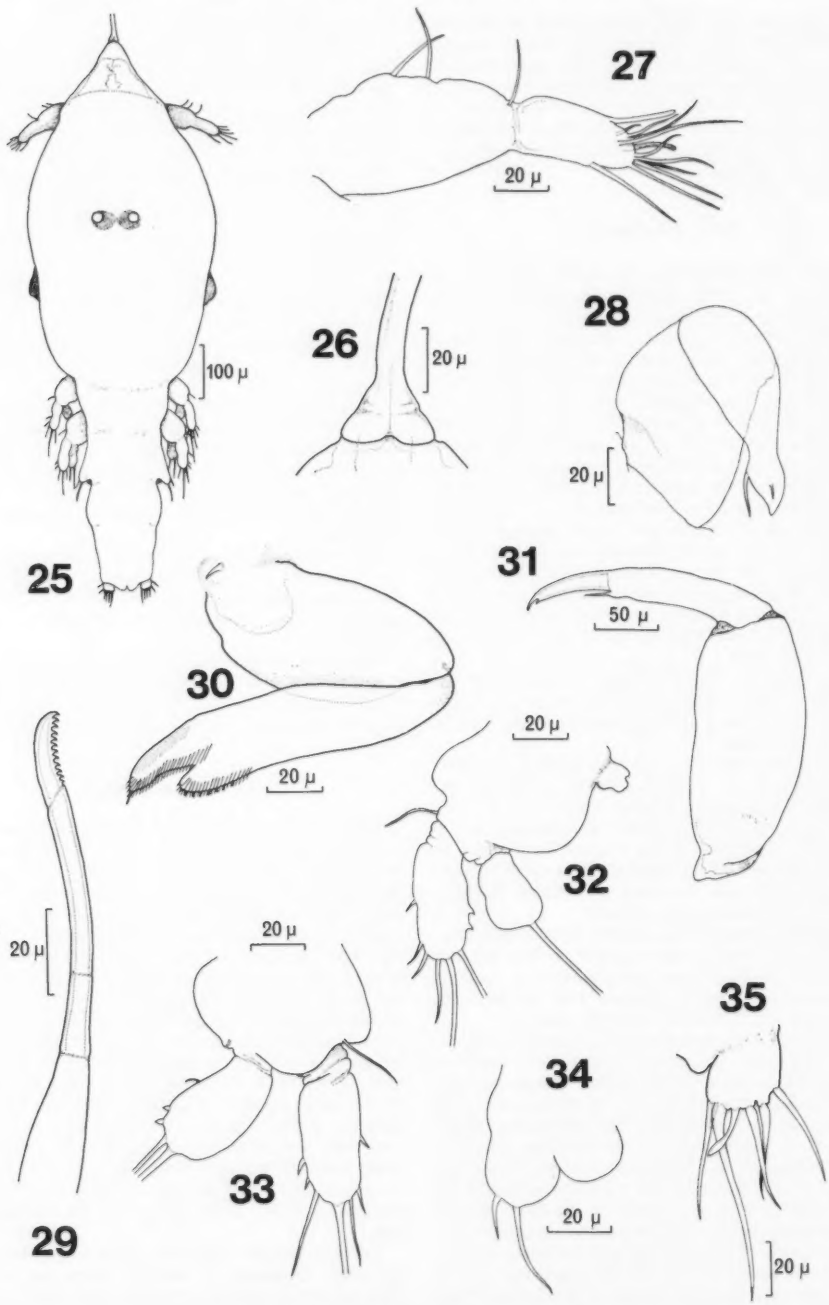
The third leg (Fig. 34) has attained a form of two hemispherical swellings, representing rudiments of its future rami. The two setae, now less unequal in length and both unarmed, arise from the apex of the future exopod.

The caudal ramus (Fig. 35) has setae reduced in length and unarmed. The aesthete-like seta can be distinguished by its inflated tip, but the two comb-like structures have vanished from the ventral surface of the ramus.

Second Chalimus

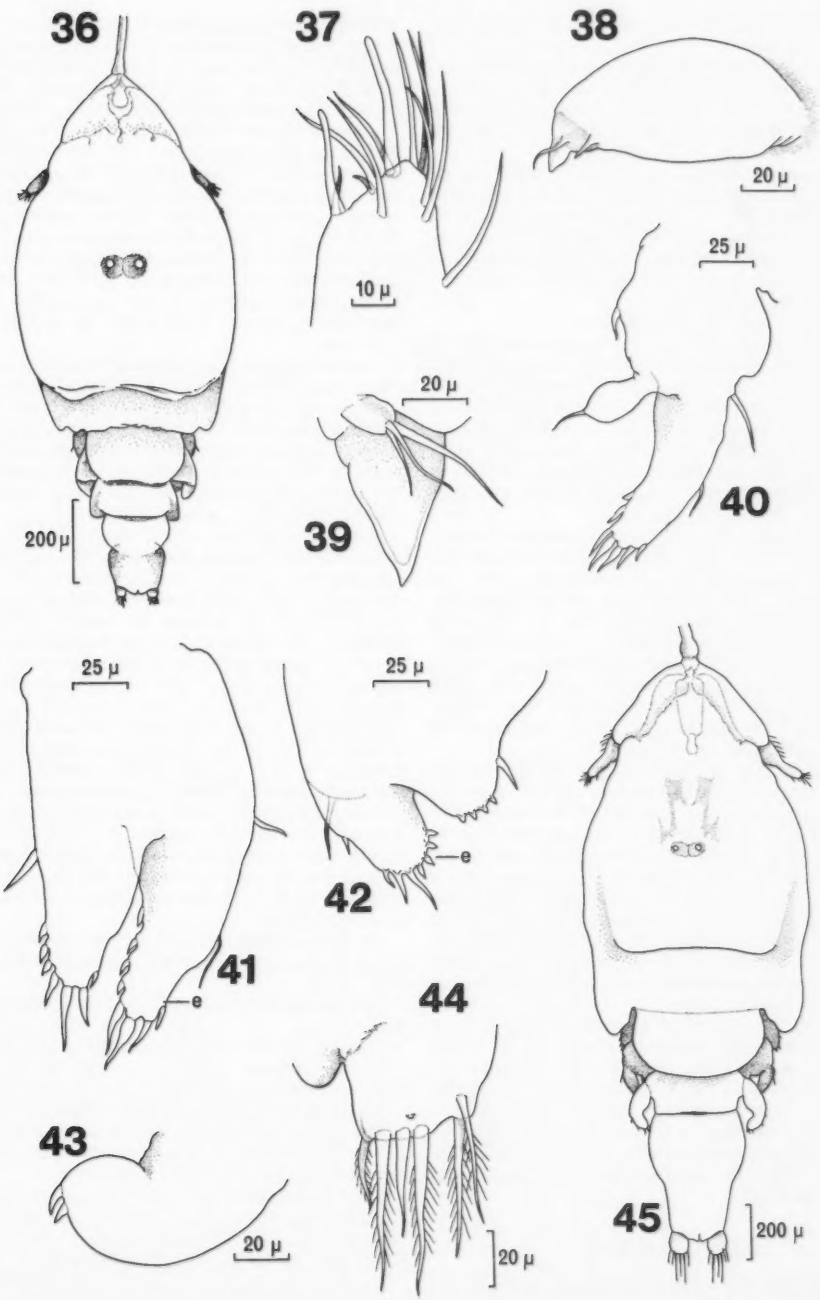
Some measure of external segmentation has been regained at this stage (Fig. 36). The cephalothorax, while retaining its general shape, is enlarged by inclusion of the third thoracic segment, i.e., that bearing the second pair of legs. Its fusion with that segment is not yet quite complete (dorsal surface of the shield bears clearly visible suture line), but both clearly belong to the same tagma. Anteriorly, rudiments of the paired frontal plates can be seen through the cuticle of the frontal sac. The posterior tagma consists of four parts. The fourth segment bears the third pair of legs. Its width is about $\frac{1}{3}$ that of the greatest width of the cephalothorax. The fifth segment now carries the fourth pair of legs. The part posterior to the fifth segment is differentiated into the genital complex (comprising two thoracic segments) and the abdomen, with ill-defined but discernible difference between them. There is a further reduction in the length of the armature of the appendages. The length (10 specimens) varies from 1.24 to 1.44 (average 1.31) mm.

The first antenna has not changed greatly from the last stage. However, its anterior margin has now eight unarmed setae, increasing in length from the base towards the tip of the appendage, the longest being at the boundary between the basal and the terminal segments. The



FIGURES 25–35. *Caligus clemensi*, first chalimus.

FIG. 25. First chalimus, dorsal; FIG. 26. Base of frontal filament, dorsal; FIG. 27. First antenna, ventral; FIG. 28. Second antenna, ventral; FIG. 29. Mandible, lateral; FIG. 30. Second maxilla, ventral; FIG. 31. Maxilliped, ventral; FIG. 32. First leg, ventral; FIG. 33. Second leg, ventral; FIG. 34. Third leg, ventral; FIG. 35. Caudal ramus, ventral.



FIGURES 36–45. *Caligus clemensi*, second and third chalimus.
FIG. 36. Second chalimus, dorsal; FIG. 37. First antenna, tip, ventral; FIG. 38. Second antenna, ventral; FIG. 39. First maxilla, ventral; FIG. 40. First leg, ventral; FIG. 41. Second leg, ventral; FIG. 42. Third leg, ventral; FIG. 43. Fourth leg, ventral; FIG. 44. Caudal ramus, ventral; FIG. 45. Third chalimus, dorsal. (e, exopod).

tip of the latter (Fig. 37) now possesses the full complement of 13 setae.

The second antenna (Fig. 38) has been further simplified. It is now unsegmented, ovoid, and ends in a short, poorly delimited claw. The latter is armed with two setae, a longer one at midlength and a shorter one near the base.

The postantennary process is absent. There are no changes in the structure of the mouth cone or mandible. The first maxilla (Fig. 39) is more like that of the adult in the relative proportions of its process and papilla.

The second maxilla remains in the same condition as it was in chalimus I. The only change in the maxilliped is its reduction in size.

There is no trace of a sternal furca.

The first leg (Fig. 40) remains unsegmented but its shape has been significantly altered by reduction in size of the endopod and corresponding elongation of the exopod. The sympod retains its lateral and medial setae. The unsegmented exopod carries one slender seta on its lateral margin, five more sturdy ones on the distal, and two dentiform ones on the medial margin. The endopod also retains its single apical seta, though it is much shorter than in the preceding stage.

The second leg (Fig. 41) is also unsegmented. Its sympod is armed with a seta on the lateral margin. The exopod (e) is equipped with nine unarmed setae. The lateral margin carries a flagelliform seta at about midlength and a short, thick one near the tip. The distal margin carries three sturdy setae of unequal length, whereas on the medial margin there are four similar, but shorter, setae. The endopod is armed with seven setae, four on the distal and three short ones on the medial margin. In addition, higher on that margin it has also a long and robust seta.

The third leg (Fig. 42) has developed greatly since the preceding stage. It is unsegmented, short and broad, with prominent rami. The armature of the sympod consists of one seta on the lateral margin near the base of the exopod. The exopod (e) is unsegmented and armed with one short seta near the middle of its lateral margin and seven more on or near the tip. Of these, three lateral ones are longer and more slender than the medial three that are uniformly short and sharp pointed. The armature of the much shorter endopod consists of four short, almost conical apical setae and one longer seta near the base on the medial margin.

The fourth leg (Fig. 43) is uniramous, being a short cylindrical outgrowth, folded at about $\frac{2}{3}$ of its length and ending in two short, peg-like, unarmed setae.

The caudal ramus (Fig. 44) has retained its six setae. All of them are now short and pinnate, except the aesthete, which is unarmed but now also with a fine tip. Above its surface a small cuticular fold is present in the position occupied in the copepodid by one of the comb-like structures.

Third Chalimus

The sexes can now be distinguished by external appearance. The male differs from the female by having a slightly slimmer genital region and by the structure of its second antenna. Progress has been made towards incorporation of the fourth thoracic segment into the cephalothorax. Although the line of demarcation between it and the preceding segment is still noticeable, the fusion is nearly complete. Anteriorly, the frontal plates have become discernible (particularly at their lateral corners). The lateral margins of the cephalothorax have developed posteriorly protruding lobes. The fourth thoracic segment provides a further extension of the dorsal shield and the medial borders of the posterior sinuses which are now apparent. The fifth thoracic segment, carrying the fourth pair of legs, is firmly separated from the genital complex. The latter is somewhat orbicular and distinguishable from the abdomen only by a slight constriction in the lateral margins of the tagma (Fig. 45). The length (10 specimens) ranges from 1.20 to 1.44 (average 1.31) mm.

The first antenna (Fig. 46 and 47) resembles that of the adult, except for the number and size of the setae on the anterior margin of the basal segment. There are 18 of them, all unarmed. The longest, at the junction of the terminal segment, reaches about $\frac{2}{3}$ of the length of that segment. The posterior margin of the terminal segment has now a long slender seta. The apical armature has attained definitive condition.

The second antenna of the female (Fig. 48) has not changed much since the preceding stage. Delimitation of the claw from the rest of the appendage is still indistinct and the two setae on the claw are shorter and less slender. At the base of the antenna the ventral surface of the cephalothorax carries on it a flat rounded sclerite (Fig. 48, r?), probably the future posterior process of its basal segment. The second antenna of the male (Fig. 49) is quite different. Its future subdivisions are more clearly recognizable. At the base there is a small sclerite (r?), similar to that of the female appendage. The border between the basal and the second segment is indistinct, but it is at this level that the appendage flexes outwards, the second segment forming an acute angle with the basal segment. The terminal segment is armed with a small conical claw, located subterminally, and two setae, one anterior and the other proximal to the claw. In addition, there is a small nipple-like outgrowth at the base of the terminal segment.

The postantennary process has reappeared (Fig. 50) in the form of a small conical outgrowth with a pointed tip.

No changes are apparent in the mouth tube or mandible.

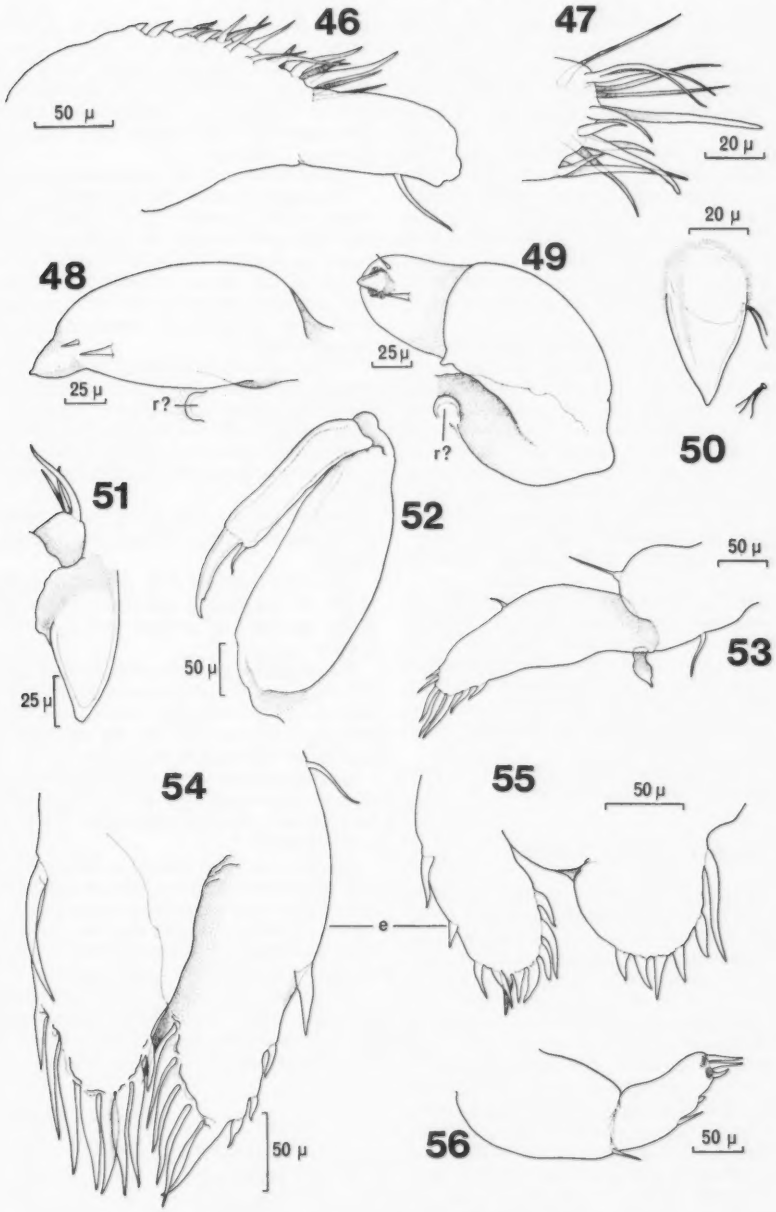
The first maxilla (Fig. 51) remains largely unchanged from the previous stage, the process being relatively small in relation to the size of the papilla.

No changes occur in the second maxilla.

The maxilliped (Fig. 52) is now fairly similar to that of the adult female. A denticle on the inner margin of the claw, though very small, is still present.

There is no sternal furca, but in its region two ridges of fairly thin cuticle diverge from a mid-ventral point obliquely backwards along what appears to be an obsolete suture line in the surface of cephalothorax.

The first leg (Fig. 53) generally resembles the adult appendage. Its endopod is further reduced and vestigial. Its exopod has become enlarged, stretching out laterally from the sympod. The latter is armed with two setae, one



FIGURES 46–56. *Caligus clemensi*, third chalimus.

FIG. 46. First antenna without apical armature, dorsal; FIG. 47. Same, tip, dorsal; FIG. 48. Female second antenna, ventral; FIG. 49. Male second antenna, ventral; FIG. 50. Female and male, postantennary process, ventral; FIG. 51. First maxilla, ventral; FIG. 52. Maxilliped, ventral; FIG. 53. First leg, ventral; FIG. 54. Second leg, rami, ventral; FIG. 55. Third leg, rami, ventral; FIG. 56. Fourth leg, ventral. (e, exopod; r?, basal sclerite of second antenna).

on the lateral and the other on the medial margin. The exopod is unsegmented. A small seta on the lateral margin marks the point of future division into two segments. The distal armature consists of seven unarmed setae, longest at the apex and decreasing in size both laterally and medially.

The second leg (Fig. 54) has become much larger than in previous stages and greatly exceeds the size of the first leg. It is still unsegmented and its sympod is armed laterally with a single seta. The exopod (e) carries four unarmed seta on the lateral margin and six longer ones on the distal and medial margin. The endopod has a long unarmed setae high on the medial margin and eight more on the distal and adjacent parts of the lateral and medial margins.

The third leg (Fig. 55) has also become much larger and begins to expand and to flatten. The two members of the pair, however, are not yet fused. Rami are still well developed, though unsegmented. The exopod (e) carries two short setae on the lateral margin. In the posterolateral corner a series of eight setae begins and extends half way up the medial margin. The endopod is rounded and its armature resembles that of the preceding stage, though it now consists of five, rather than four, setae (more like conical protuberances in the previous stage). Another large seta is present in the medial, proximal corner.

The fourth leg (Fig. 56) is two-partite. The basal part is robust and constitutes slightly more than half the length of the appendage. It is armed with a short seta in its posterodistal corner. The distal part of the leg is armed at its tip with three setae, all unarmed and two of them with rudimentary pectens at their bases. On the posterior margin there are two more setae, the proximal longer than the distal.

The caudal ramus remains similar to that of the preceding stage but all its setae are now pinnate.

Fourth Chalimus Female

In general appearance (Fig. 57) it is characterized by the presence of all the features existing also in later developmental stages. The main differences between chalimus IV and the preadult are in the proportions of various parts. The cephalothorax constitutes more than half of the body length. It is much narrower in proportion to its length than in later stages. Anteriorly the development of frontal plates has progressed and rudiments of lunules can be seen through the cuticle. The frontal filament is still attached at the mid-anterior point. The lateral margins of the shield have acquired membranous rims. The dorsal surface of the shield is now clearly divisible into cephalic, thoracic, and two lateral zones by H-shaped suture lines. (It should be noted, however, that the term cephalic zone is purely arbitrary, since the division between the thoracic and cephalic zones is marked by the position of the transverse suture that originally divided the first pedigerous (i.e. second thoracic) from the second pedigerous (i.e. third thoracic) segment.) All traces of suture dividing the second pedigerous from the third pedigerous segment have disappeared. The posterior sinuses are fully developed. The fifth thoracic (i.e. fourth pedigerous) segment is well delimited from the genital complex. The latter is broader than long and has rounded posterolateral corners. There is no fifth leg. The genital complex is firmly delimited from the abdomen. The latter is one-segmented and subquadrate. The length (10 specimens) varies from 2.76 to 3.10 (average 2.94) mm.

The first antenna has reached its definitive condition.

The second antenna (Fig. 60) is unsegmented but much longer than in chalimus III. It is flexed laterally from its base and tapers towards the tip. The position of the seta, occurring at about midlength, marks the future base of the hook, the second and smaller seta is homologous with that occurring on the hook itself in adults. The basal sclerite has not yet been completely incorporated into the appendage.

The postantennary process (Fig. 61) is now longer and is beginning to resemble that of the adult. On the medial side of its base a subspherical swelling is developing. Two fimbriate setae can now be seen at the base, a third some distance from it on the surface of the body.

The mouth cone and mandible resemble those of the adult.

The first maxilla (Fig. 62) is distinguished by the size of its process, much enlarged in comparison with the papilla.

The second maxilla (Fig. 63) has not changed much except for the relative elongation of the two processes at the tip. Both are fringed with a row of short, stiff bristles.

The maxilliped (Fig. 64) remains much as in the preceding stage, but a small subterminal denticle disappeared from the inner margin of its claw and a small dentiform outgrowth has appeared on the subchela at some distance from the base of the claw.

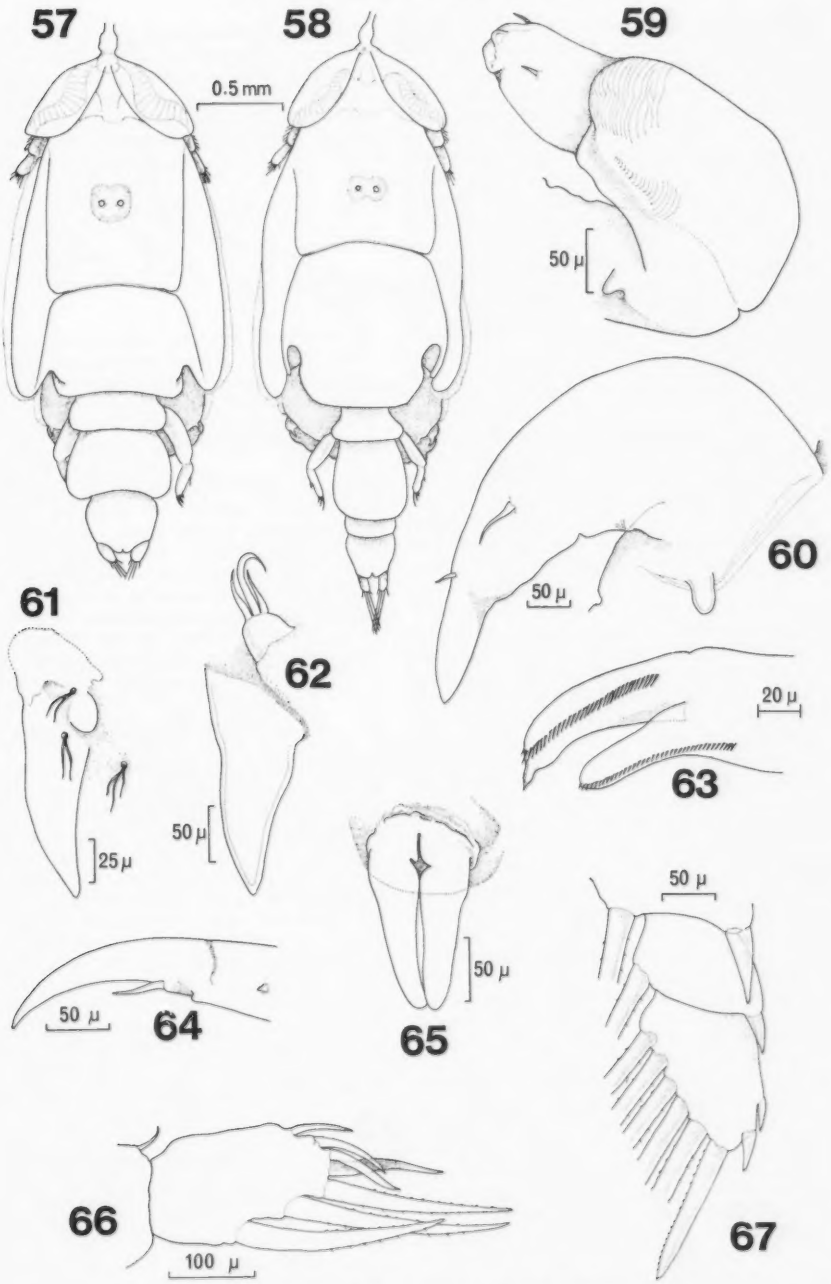
The sternal furca (Fig. 65) has reappeared. It consists of two rather soft and easily disarranged tines with rounded tips, placed close together. The box of the furca is not apparent.

The first leg (Fig. 66) has a two-segmented exopod. The armature of the distal segment is beginning to differentiate into three large pinnate setae (pinnules are represented by minute prickles) and four distal setae, all still single and unarmed. The anterodistal corner of the basal segment is armed with a small seta.

The second leg (Fig. 67 and 68) has three-segmented rami and closely resembles the adult condition. There are no membranes on the sympod and the exopod. The armature of the rami is as follows:

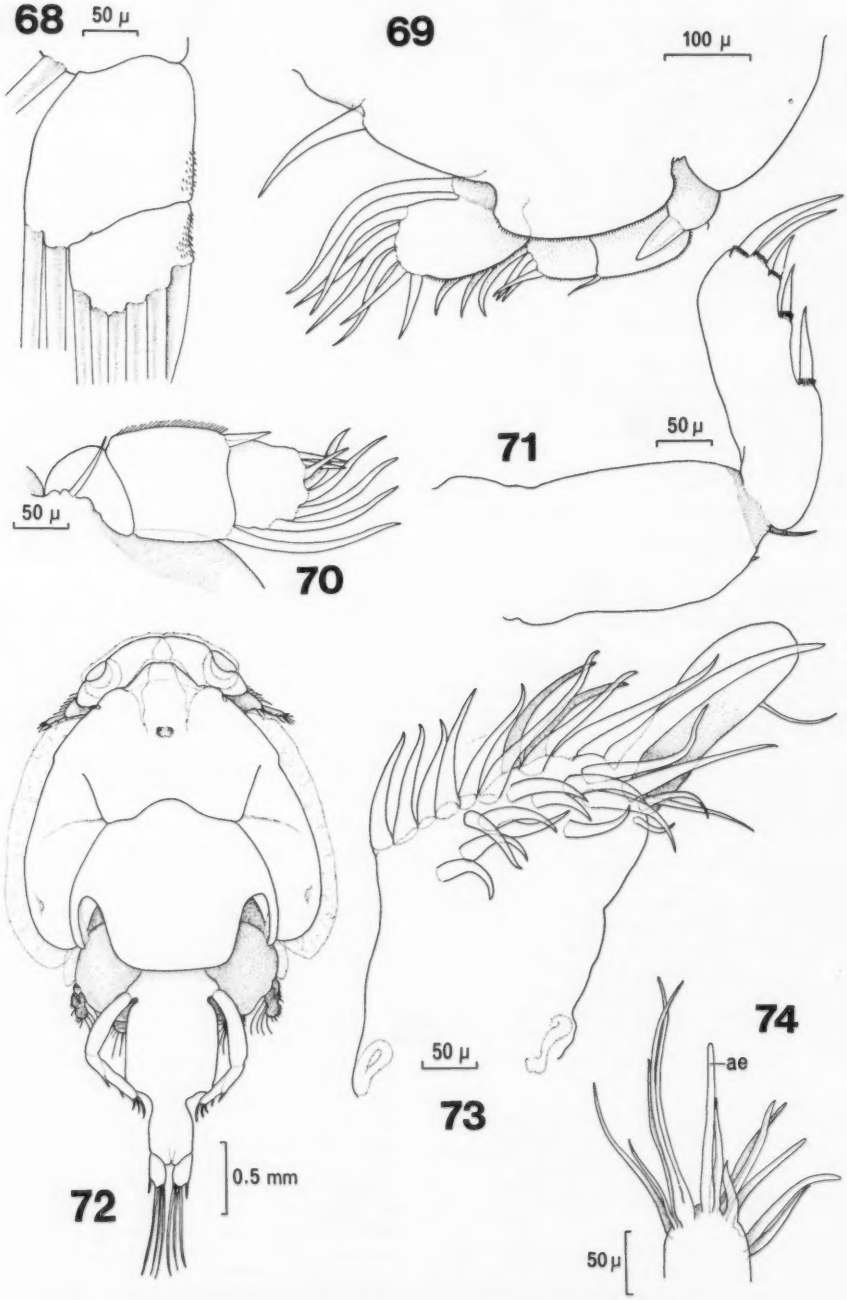
	Endopod	Exopod
Segment 1	1–0	1–I
Segment 2	2–0	1–I
Segment 3	6	6,II

The pinnate setae have rudimentary pinnules; the lateralmost seta of the exopod is armed with one medial row of rudimentary pinnules. The lateral margin of the second and third endopod segments are covered with patches of setules.



FIGURES 57–67. *Caligus clemensi*, fourth chalimus.

FIG. 57. Female, dorsal; FIG. 58. Male, dorsal; FIG. 59. Male second antenna, ventral; FIG. 60. Female second antenna, ventral; FIG. 61. Female and male, postantennary process, ventral; FIG. 62. First maxilla, ventral; FIG. 63. Second maxilla, tip, ventral; FIG. 64. Maxilliped, claw, ventral; FIG. 65. Sternal furca, ventral; FIG. 66. First leg, tip of exopod, ventral; FIG. 67. Second leg, tip of exopod, ventral.



FIGURES 68–74. *Caligus clemensi*, fourth chalimus and preadult.
FIG. 68. Fourth chalimus, second leg, tip of endopod, ventral; FIG. 69. Third leg, rami, ventral; FIG. 70. Same, exopod, dorsal; FIG. 71. Fourth leg, ventral; FIG. 72. Preadult female, dorsal; FIG. 73. Female and male, first antenna without apical armature, ventral; FIG. 74. Same, apical armature, dorsal. (ae, aesthete).

The third leg (Fig. 69 and 70) shows most of the features present in adult stages. The sympod is now flat and very broad, distally developing a velum, a sclerite that fills the space between the bases of the rami. The rim of the velum, as well as the lateral margins of the rami, are fringed with rows of rudimentary setules. The armature of the rami is as follows:

	Endopod	Exopod
Segment 1	1–0	1–I
Segment 2	6	1–I
Segment 3	–	4,III

This armature has the same spine:seta formula as the adult. The difference between them is in the less well-developed spines of the chalimus stage, particularly on the third segment of the exopod, where the differences between spines and setae are not yet clear. The basal segment of the exopod has a well-developed hook and a small seta on the dorsal side.

The fourth leg (Fig. 71) differs from the adult appendage only in details of its armature. The proximal part bears a short and a longer seta in its posterodistal corner. The distal part has three terminal and two posterior setae, unarmed but with pectens at their bases.

The appendages on the ventral surface of the posterolateral corners of the genital complex are similar to those of the next stage and will be described with it.

The caudal furca is also similar to that of the next stage but its setae are relatively shorter.

Fourth Chalimus Male

In general appearance (Fig. 58) it resembles the female very closely, particularly in its anterior tagma. Differences are evident only in the posterior tagma. The genital complex is longer than wide and more slender than that of the female, whereas the abdomen is divided into two segments, the first half as long as the second. At this stage the male specimens tend to be larger than the females of the corresponding stage. The length (10 specimens) varies from 2.88 to 3.40 (average 3.15) mm.

The appendages are like those of the female, with the exception of the second antenna (Fig. 59). The latter resembles that of the male of the preceding stage. Its basal segment has incorporated into it the small basal sclerite. The second segment has rudimentary adhesion pads on its surface.

The caudal setae are relatively longer than those of the female.

Preadult Female

In general appearance (Fig. 72), it differs from that of the adult only in the relatively poorly developed genital complex. The frontal plates are well developed, capable of articulation with the dorsal shield of the cephalothorax, and provided with functional lunules. The marginal membrane is broad and has a ventral surface equipped with densely crowded fine setae near its border with the shield. A part of the ventral surface of the shield is also covered with similar setae. The posterior margin of the thoracic zone now protrudes some distance beyond the posterior limits of the lateral lobes. The divisions between the fifth thoracic (i.e. fourth pedigerous) segment and the genital complex, as well as between the latter and the abdomen, are not marked by clear cuticular grooves. The genital complex itself is almost square and about as broad as the fifth thoracic segment, its posterolateral corners are either rounded or almost angular. The abdomen is short, its lateral margins parallel to each other.

The proximal segment of the first antenna (Fig. 73 and 74) is strongly armed along its anterior margin and a part of its ventral surface adjacent to that margin. In all, there are 26 setae, all plumose except for two that arise from the dorsal surface (shaded in Fig. 73). One of the plumose setae is longer than the distal segment and usually extends along its ventral surface. The distal segment is slightly pedunculate and bears one seta on its posterior margin. The apical armature (Fig. 74) consists of two groups of setae, distinct from each other. The posterior group comprises five long and slender setae, two of them arising from a common base. The anterior group includes the apical aesthete (ae) and seven other setae, generally shorter and stouter than those of the posterior group. This type of apical armature is characteristic for all species of the genus *Caligus* and related genera.

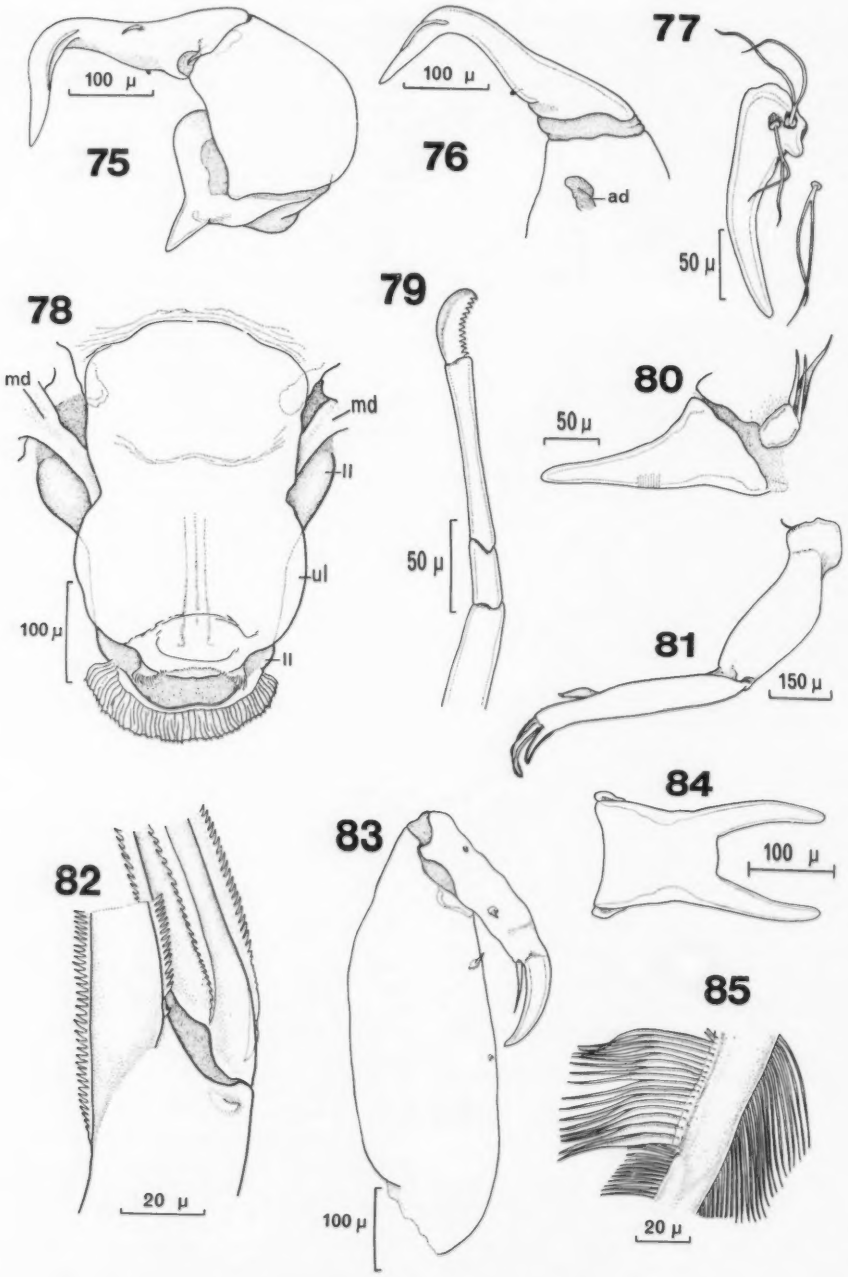
The second antenna (Fig. 75 and 76) appears to be three-segmented. The basal segment has a well-developed posterior spiniform process. The middle segment is powerfully built. On its dorsal side near the distal end there is a small adhesion pad with corrugated surface (Fig. 76, ad). The claw is sharply bent. In the region of its flexion, short narrow flanges run along the ventral and dorsal side of the claw. Two fairly short setae and one small, blunt outgrowth on the concave margin conclude the armature of the claw.

The postantennary process (Fig. 77) is long and slender, curving gently in the medial direction. On the medial side of its base there is a conical prominent swelling. At that swelling there are two long, branching setae. Another, similar seta arises from the surface of the cephalothorax some distance from the base.

The mouth tube (Fig. 78) consists of an upper (ul) and lower lip (ll) distally fused but separate at the base. The lateral margins of the lower lip are grooved proximally to provide a guide for the mandibles (md), which enter the tube through the opening between the lips. Both lips have distal marginal membranes, that of the lower lip being both longer and broader than that of the upper. The mandible (Fig. 79) has not altered from the condition it had attained during chalimus I stage.

The first maxilla's process (Fig. 80) is now much longer than the papilla. It is fairly straight and tapers from a broad base to a rounded tip. On the medial margin at about midlength it is marked by several transverse ridges extending for less than half of its circumference.

The second maxilla's characteristic features (Fig. 81 and 82) are the diminished size of the membrane on the anterior margin of the middle part (Fig. 81) and the nature of the membranes on the terminal and subterminal processes (Fig. 82). There are now four strips of serrated



FIGURES 75–85. *Caligus clemensi*, preadult.

FIG. 75. Female second antenna, entire, ventral; FIG. 76. Same, tip, dorsal; FIG. 77. Female and male, postantennary process, ventral; FIG. 78. Mouth tube, view of upper lip; FIG. 79. Mandible, lateral; FIG. 80. Female first maxilla, ventral; FIG. 81. Female and male, second maxilla, ventral; FIG. 82. Same, detail of tip, ventral; FIG. 83, Female maxilliped, ventral; FIG. 84. Female and male, sternal furca, ventral; FIG. 85. First leg, detail of pinnate seta of exopod, ventral. (ad, adhesion pad; ll, lower lip; md, mandible; ul, upper lip).

membrane on the terminal process, running its full length. Only two are present on the shorter subterminal process.

The maxilliped (Fig. 83) is distinguished by its armature and the shape of its claw. The medial margin of the appendage carries distally a short seta and proximally a minute prickle. The claw of the subchela is sharply curved and tapering. The length of the auxilliary seta is greater than half of that of the claw. There are two setae on the ventral surface of the subchela, one long and distal, the other very short and near the base.

The sternal furca (Fig. 84) has sturdy, slightly curving tines (without membranous flanges) with rounded tips. The box of the furca is longitudinally rectangular, somewhat wider anteriorly.

The sympod of the first leg (Fig. 85–87) is distinctly two-segmented, its proximal segment armed with a small seta in the distolateral corner, whereas its distal segment carries one seta on the posterior margin and another on the lateral, at the junction with the exopod. The endopod is vestigial. The exopod is two-segmented. The distal segment carries on its posterior margin three long pinnate setae (pinnules ommitted in Fig. 86), their long axes at right angles to those of the setae of the distal margin. They are armed with two types of pinnules (Fig. 85), a short proximal part of the lateral margin carrying longer, flat pinnules. The distal margin is armed with four setae (Fig. 87); the first is the shortest and unarmed; the second and third are bifid, one of their branches simple, the other carrying a strip of serrated membrane along one margin; and the fourth is the longest and unarmed.

The second leg (Fig. 88–90) has the same setae:spines formula as in the preceding stage. Flaps of dorsal membrane (Fig. 88, m) are present on the anterior margin of the sympod and on the first segment of the exopod. The lateral margin of the endopod (Fig. 88) and the lateral armature of the exopod are fully differentiated. The spines on the first and the second exopod segment have strips of membrane along their lateral margins (Fig. 89). The two lateral spines of the third exopod segment (Fig. 90) are now unequal in size (cf. Fig. 67), the distal being much larger than the proximal and characteristically rounded at its tip.

The third leg (Fig. 91) is completely fused with its opposite number by antero-posterior expansion of the interpodal bar. The ventral surface of the obscurely segmented sympod carries two batches of denticles near the base of the exopod. The lateral margin of the sympod is armed with a poorly developed ridge of transverse corrugations and two strips of marginal membrane, one on the lateral margin next to the base of the exopod, the other on the medial margin between the base of the endopod and the medial pinnate seta. The velum (Fig. 91, v) is now fully developed. The rami are similar to those of chalimus IV but their pinnate setae now have their definitive armature.

The pectens at the bases of the spines of the fourth leg (Fig. 92–94) have attained their definitive structure (Fig. 92). The first spine of the distal end has a serrated membrane along one margin, whereas the remaining spines have two strips of similar membrane (Fig. 94). The posterodistal corner of the proximal part of the leg (Fig. 93) has one short plumose seta and a small unarmed setule.

The fifth leg (Fig. 95) is represented by three setae arising directly from the ventral surface of the posterolateral corner of the genital complex. Two of them are close together and at some distance from the third.

The caudal ramus (Fig. 96) has nearly parallel lateral and medial margins. Its distal margin is uneven, receding on the lateral side. Three apical setae are long and pinnate, the medialmost of them (Fig. 96, y) carrying three rows of pinnules, the others only two rows. One small pinnate seta is located on the medial side of the three large ones, one in the distolateral corner, and one on the dorsal surface of the ramus. A short fringe of marginal setae arms the distal half of the medial margin.

Preadult Male

The cephalothorax closely resembles that of the female (Fig. 97). The genital complex is ovate, in its broadest part barely broader than the fifth thoracic segment. The abdomen is two-segmented, the first segment being shorter than the second and slightly broader.

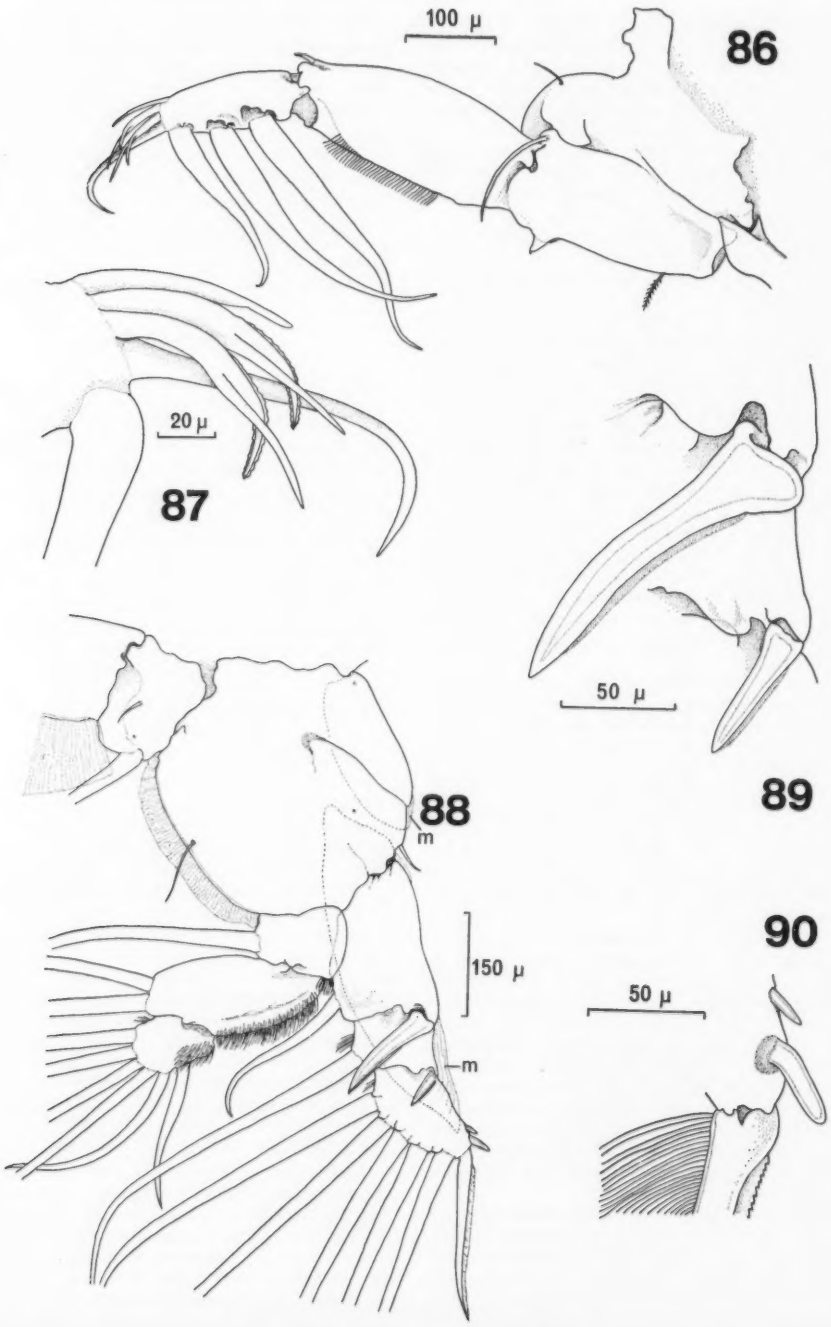
The appendages of the male are similar to those of the female, except for those described below.

The second antenna (Fig. 98–100) has a fully developed terminal claw and its middle segment is partly covered by large adhesion pads. Seen from the posterior side (Fig. 99), the tip of the claw is covered with two overlapping flanges of cuticle, a third one running along the posterior side but ending short of the tip. Near the proximal end of that flange, a long and robust but flexible seta is present. Under the hood formed by the first two flanges (Fig. 100) the claw has two tips, the distal being slightly bifid at the tip.

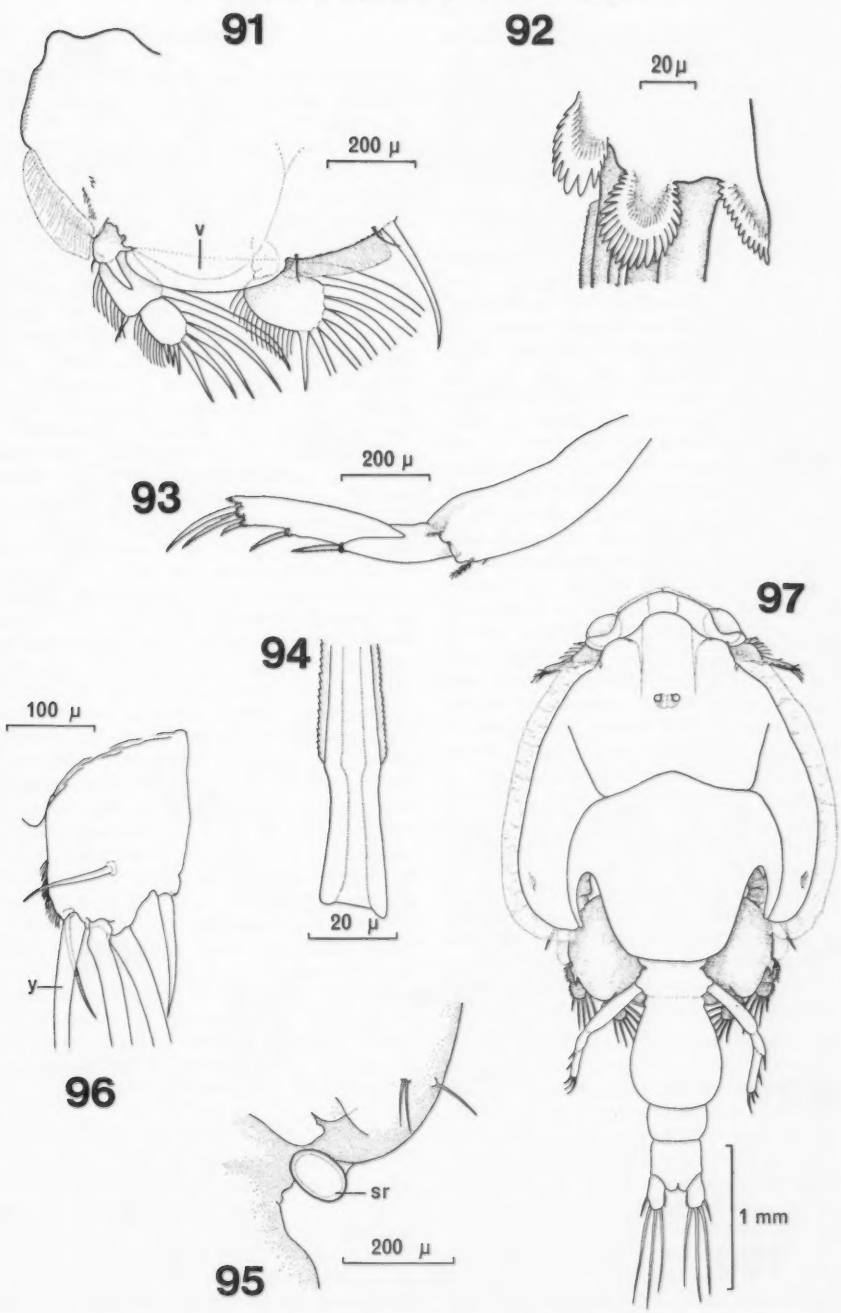
The first maxilla (Fig. 101) is generally similar to that of the female but has no transverse striations.

The maxilliped (Fig. 102–105) is broader and relatively stronger than that of the female. The distal part of its medial margin is extended in the form of a subtriangular flange (Fig. 102) which provides support for the tip of the claw when closed. The inner surface of the flange has on it a patch of rough denticles (Fig. 103). Its proximal margin has on it a strip of thinner cuticle, serrated and covered with striations, whereas the distal part of the margin is produced into a long, spiniform process (Fig. 104). Just below the flange, the margin of the maxilliped carries a short spiniform process surmounting a conical papilla (Fig. 104). The outer margin of the claw is transversely striated (Fig. 105) and its auxiliary seta is very long. Demarcation between the claw and the subchela is not very distinct (Fig. 104); there are two setiform outgrowths on the surface of the latter, one larger and distal, the other smaller and proximal.

The fifth and sixth legs (Fig. 106) are present on the ventral surface of the posterolateral corner of the genital complex. The fifth leg is anterior to the sixth and consists of three short pinnate setae arising from a bifid papilla. The sixth leg is represented by small unarmed setules. Only two such setules were found in my specimens, but Parker and Margolis (1964) reported the presence of a third. The difference might be due to damage sustained by my male specimens.

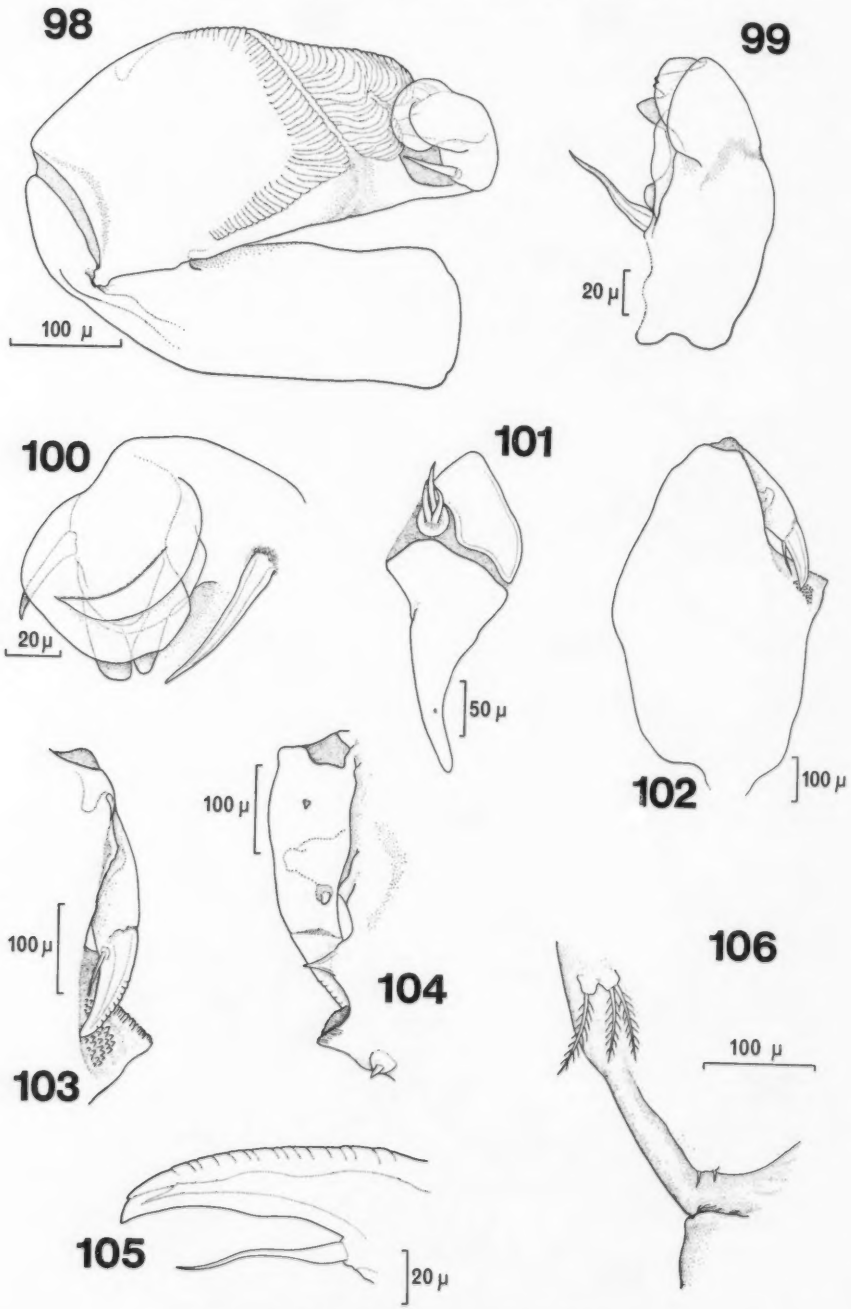


FIGURES 86–90. *Caligus clemensi*, preadult.
FIG. 86. Female and male, first leg, entire, ventral; FIG. 87. Same, tip of exopod, ventral; FIG. 88. Second leg, ventral; FIG. 89. Same, spines of second and first exopod segments, ventral; FIG. 90. Same, spines of third exopod segment, dorsal. (m, dorsal membranes of second leg).



FIGURES 91–97. *Caligus clemensi*, preadult.

FIG. 91. Female and male, third leg, ventral; FIG. 92. Fourth leg, bases of terminal spines, ventral; FIG. 93. Same, entire, ventral; FIG. 94. Base of proximal spine, ventral; FIG. 95. Female, posterolateral corner of genital complex, ventral; FIG. 96. Female and male, caudal ramus, ventral; FIG. 97. Male, dorsal. (sr, spermatophore; v, velum; y, seta with three rows of pinnules).



FIGURES 98–106. *Caligus clemensi*, preadult male.
FIG. 98. Second antenna, entire, ventral; FIG. 99. Same, claw, posterior; FIG. 100. Same, posterolateral; FIG. 101. First maxilla, ventral; FIG. 102. Maxilliped, entire, dorsal; FIG. 103. Same, subchela, dorsal; FIG. 104. Same, ventral; FIG. 105. Same, claw, ventral; FIG. 106. Posterolateral corner of genital complex, ventral.

Discussion

In the course of the last 40 years, only five papers have been written describing complete developmental cycles of caligid species. Comparison of these descriptions appears to suggest that the five species involved differ in their ontogeny much more widely than one would expect in view of their close taxonomic relationship. It is my view, however, that the differences are just as much due to semantic misunderstandings as they are due to real factual discrepancies. This opinion is supported by a closer comparison of the five cycles described among themselves and with that of *C. clemensi*.

Gurney (1934) described the development of *Caligus centrodonti* as consisting of two nauplius stages, one copepodid stage, and four chalimus stages, followed by the adult. The nauplii and the copepodid differ from those of *C. clemensi* in details attributable to specific characteristics but they clearly represent the corresponding stages in that species. Chalimus I appears slightly more advanced than that of *C. clemensi* (Fig. 25) in having expanded posterolateral lobes of the cephalothorax and in more distinct segmentation of the posterior tagma. Chalimus II also seems slightly more advanced. Its third pedigerous segment is more completely developed than that of *C. clemensi* (Fig. 36) and a fifth pair of legs is present. In some specimens the postantennary process might appear at this stage. Segmentation has almost reached its definitive stage at chalimus III. The typical H-shaped suture lines are faintly marked on the dorsal surface of the cephalothorax and postantennary processes are present in all specimens. (It is at this stage that they appear in *C. clemensi*.) The second antennae, which in *C. clemensi* are readily identifiable as male or female in chalimus III, in *C. centrodonti* show no signs of dimorphism. The chalimus IV stage of *C. centrodonti*, on the other hand, has much better developed lunules than that of *C. clemensi*. In both, the sternal furca appears at this stage. The sexes can be told apart in *C. centrodonti* by the shape of the maxillipeds, but their second antennae remain indistinguishable. In *C. clemensi* the situation is opposite. According to Gurney, the next stage is a full adult.

Heegaard's (1947) interpretation of the developmental cycle of *Caligus curtus* is at variance with all others described so far in that it postulates the existence of two copepodid stages and a pupal stage preceding chalimus I. He found five chalimus stages in all, followed by the adult. Examination of Heegaard's drawings of the pupa shows this stage, removed from enclosing copepodid exuvium, to be comparable in its development to chalimus I of other species, *C. clemensi* included. In fact, its appendages, and particularly its first legs, are more advanced than those of chalimus I stage of *C. clemensi*. Heegaard's chalimus II corresponds with chalimus III of other authors and his chalimus III with IV of other authors (witness appearance of H-shaped sutures and of sternal furca). A similar relationship is true of older stages.

Lewis (1963) made a thorough study of the development of a copepod belonging to a different genus *Lepeophtheirus dissimulatus*. His work is particularly valuable because it allows us to compare development of two genera of Caligidae. *L. dissimulatus* passes through two nauplii and one copepodid stage. The latter is followed by six chalimus stages and the adult. The first four chalimus stages are similar to those of the other caligids, though they differ in details. For example, *L. dissimulatus*, unlike *C. clemensi*, has the fourth legs present at chalimus I stage. The other legs are also better developed and more clearly segmented. At chalimus II the second pedigerous segment is completely incorporated into the cephalothorax and, later during the same stage, the border between it and the third pedigerous segment becomes indistinct. On the other hand, no differentiation is present between the genital complex and abdomen. The border between them appears in chalimus III. At this stage the postantennary processes also make their appearance. On the other hand, one cannot distinguish between the sexes by differences in the second antennae. The sternal furca appears in chalimus IV. Segmentation is now complete but there is still no marginal membrane of the cephalothorax. In other respects, chalimus IV resembles that of *C. clemensi*. The stages designated as chalimus V and VI are comparable with the first and the second preadult stages of Izawa (1969). The former is still found attached by the frontal filament, but becomes detached before moulting into the next stage. The moult, vividly described by Lewis (1963), occurs while the animal is forced to maintain its hold on the host with its prehensile appendages only.

Hwa (1965) studied the development of *Caligus orientalis*. As regards pre-chalimus stages, the only difference between his description and others is the use of the term metanauplius for the second nauplius stage. The copepodid is followed by five chalimus stages and the adult. Chalimus I is distinguished by more definite segmentation of the posterior tagma but is otherwise as in *C. clemensi*, except for the possession of postantennary processes, appendages which do not appear in *C. clemensi* until chalimus III. Chalimus II of *C. orientalis* has its third pedigerous segment included into the cephalothorax, but again largely resembles *C. clemensi* of the same stage. The fifth leg and a

narrow strip of cuticle at the site of the future furca appear at chalimus III. The frontal plates are well advanced in development. The furca in a rudimentary form appears in chalimus IV. The marginal membrane is now present and the abdomen has become two-segmented. Male and female second antennae are distinguishable. Chalimus V closely resembles the preadult of *C. clemensi*.

Finally, the development of *Caligus spinosus* was described by Izawa (1969). Beginning with two nauplius and one copepodid stages, the cycle passes through three chalimus and two preadult stages. Chalimus I resembles very closely the corresponding stage of *C. clemensi*. Chalimus II differs from its counterpart in that species by more advanced development of the cephalothoracic shield, particularly its posterolateral lobes. At chalimus III stage this similarity is maintained. Postantennary processes appear, but the second antennae of the males and the females are still indistinguishable. The following stage is the first preadult, again very much like that of *C. clemensi*. A definite gap is thus created in the development of the cephalothorax, particularly in that of the lunules, sternal furca, and the pinnate setae.

Of the six species compared, one (*C. centrodonti*) is reported to have eight development stages, adult included; three (*C. orientalis*, *C. spinosus* and *C. clemensi*) are reported to have nine stages; one (*L. dissimulatus*), ten stages; and one (*C. curtus*), eleven stages.

Clearly, the first three stages of all species (up to the copepodid) are homologous and comparable. The first difficulty arises with Heegaard's second copepodid and pupa. In describing these stages, however, Heegaard might have been misled by the ontogenetic events, particularly in view of the fact that he did not actually see the moult separating the first copepodid from the alleged second (Heegaard 1947). As stated earlier, his pupa was probably chalimus I in the process of moulting, before the cuticle of the copepodid had been shed. From the morphological point of view it is easy to equate Heegaard's pupa with chalimus I of other authors. Hence his chalimus III is, in fact, chalimus IV. The next difficulty arises at the chalimus IV stage, apparently absent in *C. spinosus*. Izawa (1969) describes the stage following chalimus III as the first preadult. The preadult condition is clearly distinct from that of the chalimus, regardless of its stage, by the existence of the definitively developed sternal furca and by presence of fully pinnate setae on the swimming legs. Also, preadults are free-moving, the first preadult stage breaking away from its attachment to the frontal filament. Since Izawa's chalimus III has been correctly diagnosed and since his first preadult is also what that author claims it to be, one must conclude that the chalimus IV stage has either been jumped over in the development cycle or missed by the author in its study. If, as is probable, the latter is true, then all the species discusse d here have four chalimus stages. Difficulties arise at the preadult stages. None was described by Gurney (1934), one by Heegaard (1947) and Hwa (1965) (both as chalimus V), as well as by myself, two by Izawa (1969) and Lewis (1963) (the latter referred to them as chalimus V and VI). Hewitt (1964) suggested that three postchalimus stages occur in *Lepeophtheirus polyprioni* but provided no evidence that they are separated from one another by moults. Clearly, more work is needed to clear up the situation. I am inclined, however, to accept that Izawa and Lewis are correct. Unless one watches the experimental animal continuously, especially when that animal is capable of changing its place on the host, one is liable to miss a moult quite easily. When one adds to it the fact that moults, according to Heegaard (1947), tend to take place in the small hours of the morning, when the animal is less likely to be under careful surveillance, it is not impossible to visualise that a gap might occur in observations at that stage. (It might be worth adding that recent work by Voth (1972), not yet published in full, describes ten stages in the life cycle of *Lepeophtheirus hospitalis*. These stages correspond exactly with those described by Lewis (1963) for *L. dissimulatus*. Interestingly, the postantennary processes make their appearance at the chalimus III stage and the sternal furca at the chalimus IV stage.)

In summary, therefore, I suggest that, in accordance with the data at present available, all caligids have developmental cycles comprising five phases and ten distinct stages. The phases are those of nauplius (two stages), copepodid (one stage), chalimus (four stages), preadult (two stages), and adult.

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I wish to express my gratitude to Dr R. R. Parker, Pacific Biological Station, Nanaimo, who reared *C. clemensi*, collected a series of developmental stages, and kindly placed it at my disposal. My gratitude is also due to Mr B. Cousens, Zoology Department, University of Victoria, who supplied me with additional nauplii and chalimus I specimens, and to Dr L. Margolis, Pacific Biological Station, Nanaimo, whose interest and encouragement greatly contributed to this work.

GURNEY, R. 1934. Development of certain parasitic copepods of the families Caligidae and Clavellidae. Proc. Zool. Soc. (London) 1934: 177–217.

HEEGAARD, P. 1947. Contribution to the phylogeny of the arthropods, Copepoda. Spolia Zool. Mus. Hauniensis Kobenhavn 8: 1–236.

HEWITT, G. C. 1964. The postchalimus development of *Lepeophtheirus polyprioni* Hewitt, 1963 (Copepoda: Caligidae). Trans. Roy. Soc. N.Z. 4: 157–159.

HWA, T.-K. 1965. Studies on the life history of a fish-louse (*Caligus orientalis* Gusev). Acta Zool. Sinica 17: 48–57. (In Chinese with English summary)

IZAWA, K. 1969. Life history of *Caligus spinosus* Yamaguti, 1939, obtained from cultured yellow tail, *Seriola quinqueradiata* T. & S. (Crustacea: Caligoida). Rep. Fac. Fish. Prefect. Univ. Mie 6: 127–157.

KABATA, Z. 1970. Crustacea as enemies of fishes. *In* S. F. Snieszko and H. R. Axelrod [ed.] Diseases of fishes. Book 1. T. F. H. Publications, Jersey City, N.J. 171 p.

LEWIS, A. G. 1963. Life history of the caligid copepod *Lepeophtheirus dissimulatus* Wilson, 1905 (Crustacea: Caligoida). Pac. Sci. 17: 192–242.

PARKER, R. R., Z. KABATA, L. MARGOLIS, AND M. D. DEAN. 1968. A review and description of *Caligus curtus* Müller, 1785 (Caligidae: Copepoda), type species of its genus. J. Fish. Res. Bd. Canada 25: 1923–1969.

PARKER, R. R., AND L. MARGOLIS. 1964. A new species of parasitic copepod, *Caligus clemensi* sp. nov. (Caligoida: Caligidae), from pelagic fishes in the coastal waters of British Columbia. J. Fish. Res. Bd. Canada 21: 873–889.

VOTH, D. R. 1972. Life history of the caligid copepod *Lepeophtheirus hospitalis* Fraser, 1920 (Crustacea: Caligoida). Diss. Abstr. Int. B Sci. Eng. 33: 5547–5548.

Some Effects of Artificial Fertilization on Enclosed Plankton Populations in Marion Lake, British Columbia[1]

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DICKMAN, M., AND I. E. EFFORD. 1972. Some effects of artificial fertilization on enclosed plankton populations in Marion Lake, British Columbia. J. Fish. Res. Bd. Canada 29: 1595–1604.

After single applications of nutrients to enclosures in Marion Lake in each of three seasons (fall, summer, and spring), primary productivity always increased within a 2-week period whether nitrate, phosphate, or a combination of the two was used. The species that responded to the three nutrients generally differed both within and between seasons. In all seasons, there was a decrease in total plankton diversity (based on productivity) accompanied by an increase in relative productivity of a few phytoplankton species. During fall, phosphate (P) was most stimulatory, raising primary productivity 6 mg C/m^3 per hr above that of the unenriched control. During spring, N+P was most stimulatory (23 mg C/m^3 per hr) and nitrate (N) next (11 mg C/m^3 per hr). In summer, N+P was again most stimulatory (44 mg C/m^3 per hr), primary production in the untreated lake rarely exceeding 4 mg C/m^3 per hr. Of the species that increased in density, 78% were not detectable (< 1 cell/ml) at the time of fertilization. A rapid increase in density of rare species culminated in an algal bloom 2–3 weeks after fertilization.

After the first phase of succession following fertilization, there was an increase in plankton diversity. At 1.5 months after fertilization, the diversity and the number of species detected were as high as or higher than before fertilization. In addition, the number of phytoplankton species per liter did not decrease significantly after enrichment though diversity decreased sharply due to the increase in abundance of a few species.

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Nous avons mesuré les effets d'enrichissements par dosages uniques de phosphate et de nitrate sur la composition spécifique et la productivité primaire du phytoplancton dans des enclos du lac Marion, sur une période de deux mois au cours de trois saisons (printemps, été, et automne). Après chaque enrichissement, nous avons mesuré deux fois la semaine la productivité primaire, le phosphate et le nitrate dissous, la biomasse et la composition spécifique du plancton.

Les espèces planctoniques se succèdent suivant des modalités prévisibles, qui se répètent en toutes saisons et par toutes les conditions nutritives. Il y a diminution de la diversité globale du plancton et augmentation de la productivité primaire de quelques espèces phytoplanctoniques. En automne, le phosphate (P) est le plus efficace, élevant la productivité primaire de 6 mgC/m^3/h au-dessus de celle du lac témoin. A printemps, une combinaison N + P est le meilleur stimulant (23 mgC/m^3/h). Vient ensuite le nitrate (N_3) (11 mgC/m^3/h). En été, la combinaison N + P est de nouveau en tête (44 mgC/m^3/h), stimulant une production de phytoplancton qui n'excède que rarement 4 mgC/m^3/h dans le lac non traité. Dans 78% des cas, la fertilisation artificielle ne réussit pas à augmenter la productivité primaire des espèces communes de phytoplancton présentes au moment de l'enrichissement. En revanche, des espèces auparavant rares deviennent rapidement denses. Il en résulte, au point culminant, une floraison algale 2–3 semaines après la fertilisation.

Après la première phase de la succession suivant la fertilisation, il y a augmentation de la diversité du plancton. Un mois et demi après enrichissement, la diversité du plancton et

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le nombre d'espèces à l'intérieur des enclos sont aussi, et même plus élevés qu'avant la fertilisation. De plus, le nombre total d'espèces phytoplanctoniques par litre ne diminue pas sensiblement après l'enrichissement, quoique la diversité diminue brusquement par suite de l'augmentation du nombre de quelques espèces.

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EXPERIMENTAL fertilization studies provide a basis for predicting the consequences of man-induced enrichments and point out dangers which can result from interference with the "metabolism" of a body of water (Ruttner 1953). The addition of one or more nutrients originally in low concentration may initially inhibit phytoplankton productivity (Fournier 1966), may have no immediate effect because another nutrient was limiting (Einsele 1941), or may result in the rapid increase in abundance and primary productivity of one or more species (Brook and Woodward 1965). In every case, the added nutrient soon returns to its pre-enrichment concentration if no more is added.

When a nutrient is added to a lake, its initial effects are most often reflected in increased abundance of small, rapidly dividing, primary producers (Prescott 1960), which in turn may affect the physico–chemical properties of the entire body of water. For this reason, an understanding of initial effects of fertilization on species composition and bloom formation is essential before one can speculate on its secondary effects on bottom fauna and plankton.

The purpose of this study was to determine whether or not a predictable pattern occurred following lake fertilization and how such a pattern would be influenced by seasonal differences. In addition, three different nutrient regimes were used to determine whether the pattern was dependent on the type of fertilization.

Marion Lake is situated in the coastal mountains of southwestern British Columbia (49°09′N 12°33′W) 300 m above sea level. The lake is 800 m long and 200 m wide with an area of 13.3 ha. It has a drainage basin of approximately 13.0 km^2. An annual rainfall of 240 cm per year was recorded over a 10-year period (1958–68). Runoff is heavy due to the shallow soil and steepness of the drainage basin. The lake is very oligotrophic due to its high flushing rate and low nutrient content (Efford 1967; Dickman 1969). During periods of heavy runoff the lake level rises to a maximum depth of 7 m. In many respects it would be more accurate to consider the study area as a widening in a creek rather than a lake (Dickman 1969; and Swale 1964).

Materials and Methods

Experimental enclosures — Four plywood enclosures were placed in the lake at each of two stations, X and Y (Fig. 1). The design was treated as a mutually orthogonal one since each block of four enclosures contained all the treatments and a control. The enclosures were 3.05 m (10 ft) square and 1.84 m (6 ft) high, with the base set 0.60 m in the lake sediment. Since the mean depth of water at X and Y was 1.0 m when the lake was at its mean height, 0.23 m of each enclosure was exposed above the surface of the lake under these conditions; each of the eight enclosures then held approximately 6000 liters of water. Fluctuations in lake level were considerable in winter, forcing the cancellation of the fertilization

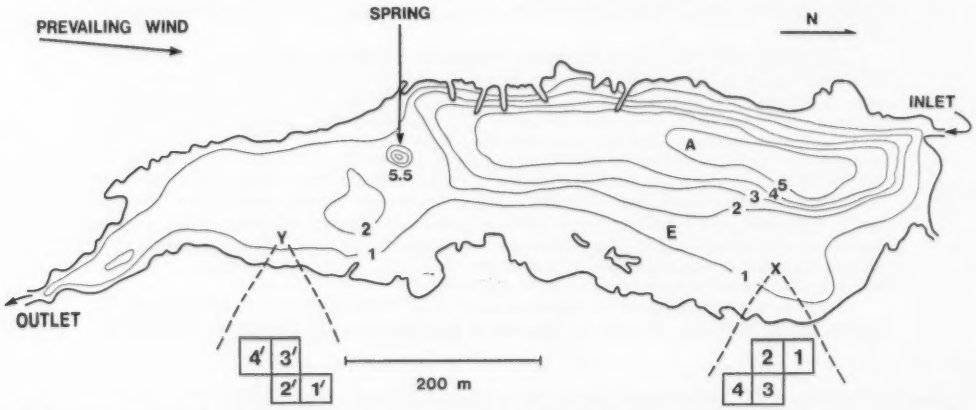

FIG. 1. Locations (X,Y) of the eight wooden enclosures to which nutrients were added, and of the external controls (A,E). Contours in meters.

series in that season. Experiments are not reported for fertilizations where the lake level rose above the tops of the wooden enclosures. Large enclosures were constructed to minimize periphyton wall effects, to permit wind mixing within them, and to minimize self shading.

Enrichment — Hutchinson (1967) states that, "It is commonly believed that the quantity of phytoplankton that can develop in any water is more likely to be determined by the concentration of combined nitrogen and of phosphorus than by any other factor." Since these two nutrients were in very low concentration in Marion Lake (Dickman MS 1968), they were chosen as the two most likely to stimulate primary productivity and bloom formation.

Calcium salts of nitrate and phosphate were chosen because calcium was the most abundant cation in the lake and therefore the least likely to introduce changes in itself. These nutrients were added at the beginning of each seasonal series (Table 1) in concentrations similar to those used by Fournier (1966).

Before chemical enrichment the enclosures were flushed with lake water using a centrifugal pump.

Sampling — A 2-liter plankton sample was taken in a Winchester bottle three times a week from all enclosures and from lake sites "A" and "E" (Fig. 1) at a depth of ½ m. Each sample was assigned a date–location code number to ensure against unconscious bias while counting the plankton. Samples were preserved with Lugol's I.K.I. solution and then concentrated by sedimentation (Dickman MS 1968). The method of sedimentation and enumeration was similar to that used by Margalef (1969).

Nutrient analysis — With each plankton sample, a second 1-liter water sample was also taken at ½ m in a polyethylene container. These samples were analyzed within ½ hr at a lakeside laboratory for nitrate, nitrite, phosphate (total and ortho), bicarbonate, and free CO_2 according to the methods described by the American Public Health Association et al. (1965). Total and orthophosphate were analyzed using the stannous chloride method and "Hach" chemicals. The "Hach" technique was not sensitive below 0.02 mg/liter total dissolved phosphate. Nitrate and nitrite were analyzed using the I. C. phenoldisulphonic acid method and "Hach" chemicals. This test was sensitive to changes in dissolved nitrate above 0.10 mg/liter. As will be seen, knowledge of nutrient concentrations below these levels is not important in establishing the conclusions reached in this study.

Nitrate and phosphate concentrations in the open lake were determined on four occasions (spring, summer, fall, and winter) at stations A and E using the method of Strickland and Parsons (1960) and a Beckman D–U spectrophotometer equipped with a 10 cm cuvette. The NO_3–N concentrations ranged between 0.26 and 2.44 μg atoms nitrogen per liter while the PO_4–P ranged between 0.05 and 0.86 μg atoms phosphate phosphorus per liter.

Standing crop — Two methods of estimating plankton standing crop were compared. The first consisted in measuring the height of the sedimented plankton in the 16-ml vials (4 dr with a 2-cm inside diameter) in which they were stored. This method was the least reliable method (Ruttner 1953, p. 143) as it makes no allowances

TABLE 1. Nutrient concentrations added (September 7, 1966; April 26, 1967; July 11, 1967) to the wooden enclosures in each of the three seasons.

Enclosure	Chemical added	Concentration (*mg-at/liter*)
Fall series, 1966 (5900 liters)[a]		
4 & 4′	$CaHOP_4 \cdot 4H_2O$	0.93
3 & 3′	$Ca(NO_3)_2$	2.04
1 & 1′	$Ca(NO_3)_2$ & $CaHPO_4$	2.04 & 0.93
2 & 2′	None	0.0035 & 0.00042[b]
A & E	None	" "
Spring series, 1967 (7000 liters)		
1 & 1′	$CaHPO_4 \cdot 4H_2O$	0.91
2 & 2′	$Ca(NO_3)_2$	2.00
3 & 3′	$Ca(NO_3)_2$ & $CaHPO_4$	2.00 & 0.91
4 & 4′	None	0.0020 & 0.00039
A & E	None	" "
Summer series, 1967 (6000 liters)		
1 & 1′	$Ca\ HPO_4 \cdot 4H_2O$	0.90
3 & 3′	$Ca(NO_3)_2$	2.00
2 & 2′	$Ca(NO_3)_2$ & $CaHPO_4$	2.00 & 0.90
4 & 4′	None	0.0048 & 0.0005
A & E	None	" "

[a]Volume of enclosures.
[b]$Ca(NO_3)_2$ and $CaHPO_4$, respectively.

for differences in compaction between differently shaped organisms, nor does it distinguish between tripton and plankton.

The second method was based on direct counts. Forty plankton samples were chosen arbitrarily, ten from each season, and the plankton counted. The average biomass of any one species was calculated after measuring the length and width of at least 25 organisms of that species and then calculating its mean volume using the formula for an appropriately shaped spheroid (Kutkuhn 1958). The number of individuals of a particular species was then multiplied by the mean volume estimate for that species. By repeating this procedure for each species present, the total biomass of the sample was estimated (Dickman 1968). When this estimate of plankton standing crop was compared with the sediment height method, the two were found to give fairly similar results (correlation coefficient 0.74 with 39 df). Consequently, the sediment height method was employed. There appear to be two reasons why the sedimentation method provided a satisfactory estimate of standing crop in Marion Lake. Tripton was not a problem because it was sparse inside the enclosures and fluctuations in its concentration were negligible. Differences in sediment height due to compaction did not appear to vary greatly between samples because over 90% of the primary producers in the lake were similarly shaped nannoplankters.

Primary productivity — Algal productivity was estimated using the radio carbon^{14} technique (Goldman 1963). A pair of 300-ml B.O.D. bottles were filled at ½-m depth in each of the enclosures and external control locations three times a week during the experiments. A similar B.O.D. "dark" bottle was filled for each of the three treatment types and both internal and external controls. Four microcuries of carbon^{14} were added to each bottle and all samples were incubated at ½-m depth at Station E. The bottles were kept in black cloth bags or inside a covered box from the time the original sample was withdrawn to the time that incubation began. The incubation period extended from 10 AM to 2 PM at which time the samples were placed in a light-tight box to stop primary productivity. A known fraction (50–100 ml) of the sample was filtered through an H. A. Millipore^{R} 0.45 μ pore diameter filter at 78 cm Hg(15 psi) pressure. The filters were then numbered and dried in a desiccator and counted by the International Agency for the Determination of Carbon-14 in Denmark.

Data analysis — Changes in density of each of the primary producers were analyzed by using a computer program. The mean densities of each species during each sampling period in a particular season were computer plotted. The plotted curve was then fitted using the least squares fit technique for second and third order polynomials (Fisher 1951). The coefficients of the standard set of normal linear equations were thus calculated and the resulting linear system of equations was solved for the coefficients of the polynomial.

The response pattern of a particular species was evaluated by comparing its changes in density with time in the treated and control enclosures. Significant differences (polynomial coefficients which deviated significantly from the controls between the two patterns) are summarized in Table 2.

The original data can be obtained from the Marion Lake Project File in the Institute of Resource Ecology at the University of British Columbia, Vancouver, B.C. The data consist of the following information: daily rainfall values (inches), inlet flow (ft^3/sec), light intensity (g-cal/cm^2 per hr), temperature (in C), light duration (the number of hours per day that the light intensity was above 0.1 g-cal/cm^2 per hr); and semi-weekly estimates of total CO_2 (mg/liter), *p*H, primary productivity (mg C/m^3 per hr), and plankton cell density (no. per liter) for each enclosure as well as stations A and E.

Diversity — Changes in relative abundance of various planktonic species following enrichment were expressed using an index of diversity (Hp) which was based on the relative productivity of each species. Initially, the index of diversity developed by Margalef (1962) was used but it proved insensitive to changes in density of the zooplankton species. We have termed this effect "swamping" because the high density of the nannoplankton and small algae meant that even a major change in density of the larger species such as ciliates and zooplankton was not reflected by Margalef's index of diversity.

In order to remedy this situation, an index (Hp) based on three factors (density, biomass, and turnover rate) was used as it eliminated "swamping" of major trophic levels (Dickman 1968). When an index (H_b) calculated on the basis of biomass alone (Wilhm 1968; Dickman 1968) was used, a biomass "swamping" occurred; the volume of the zooplankton, even though they were rare, was so large a fraction of the total sample volume that changes in biomass of smaller species were rarely reflected.

The index of diversity (Hp) was similar to the index developed by Margalef as both are based on Shannon's (1948) basic formula $\Sigma Pi \log Pi$. The difference is that Pi is here defined as pr/PR where pr is the productivity of a particular species in the sample and PR the total sample productivity (Σpr). Productivity was calculated by multiplying a species' mean sample density and biomass, as previously calculated, by the annual turnover rate for that species. Turnover rate was defined as the number of times a species reproduced per year. Thus, an algal species which was present in the plankton for 80 days and which reproduced, on the average, once every 48 hr was assigned a turnover coefficient of 40. The turnover coefficient was then multiplied by the sample density and by the mean biomass of the species. Summing partial productivity based diversity contributions (Dickman 1968) of each species in the sample resulted in an index of diversity (Hp) for that sample. Hp, which is based on relative productivity, is unlike Margalef's H, which is based solely on relative density. Hp was sensitive to changes in relative abundance of each of the major trophic levels represented in the sample. An index of community diversity sensitive to changes in relative abundance of all the trophic levels was felt to be a mandatory prerequisite of comparative community studies. For this reason Hp and not H or H_b was used

throughout this study to compare differences in the successional pattern of diversity in the variously enriched enclosures.

Results

Nutrient concentrations — As observed by many workers (e.g. Hayes et al. 1952; Phillips 1964), the dissolved nitrate and phosphate concentrations decreased rapidly following fertilization. By the end of the second week the nutrients which were added to the six treated enclosures were no longer detectable (Fig. 2). The variance for nutrient concentration during the 2 months following fertilization was appreciable owing to the small number of replicates and the tendency of the replicates to get out of phase, that is, one species would bloom earlier in the north replicate than in the south replicate or vice versa.

Effects on primary productivity — The effects of nutrient enrichment on the primary productivity within the enclosures was obscured, in part, by rainfall. In every case, rainfall, which often doubled

TABLE 2. Responses of the species whose densities changed significantly after nutrient enrichment in one or more of the three experiments.

Species	Response[a]	Season[b]	Treatment[c]	Time of response[d]
Cyanophyta				
Coelospharium sp.	+	Sp	N+P	L
	+	S	N+P	L
Microcystis sp.	–	F	P	L
Chroococcus sp.				
	–	F	N	L
	–	F	P	L
Chlorophyta				
Scenedesmus denticulatus Lag.	+	F	N+P	E
	+	S	N	E
Ankistrodesmus spiralis (Lemm)	+	S	N, N+P	M
Pediastrum tetras (Ehr.) Ralfs	+	S	N+P	M
Nephrocytium sp.	+	S	N	M
Elakatothrix gelatinosa Wille	+	S	N+P	M
Chlamydomonas sp.	+	S	N, N+P	E
Pedinomonas-like	+	S	N	E
Chrysophyta				
Chrysapsis-like	+	S	P	E
	+	S	N+P	L
Ochromonas-like	+	S	All	M
Dinobryon sertularia Ehr.	–	Sp	P	L
Dinobryon bavaricum Imhof	–	F	N	M
Cyclotella ? glomerata Kutz.	+	S	N	E
Frustulia rhomboides (Ehr.) de Toni	+	F	P	L
Synedra ? filiformis Ehr.	+	F	N, N+P	M
	+	S	N	M
Navicula ? minima Grun.	+	S	P	M
Euglenophyta				
Trachelomonas sp.	–	F	N	L
	–	S	N+P	M
Cryptophyta				
Cryptomonas erosa Ehr.	+	Sp	All	M
Cryptomonas ovata Ehr.	+	S	P	M

[a] +, increased ($P = 0.05$) in density; –, decreased.
[b] F = Fall, Sp = Spring, and S = Summer.
[c] N = Ca $(NO_3)_2$, P = Ca $HPO_4 \cdot 4H_2O$, N+P = NO_3 + PO_4, and All = N,P and N+P.
[d] E = early (weeks 1–2), M = mid (weeks 2–3), L = late (weeks 3–4).

the volume of water within the enclosures in less than a day, resulted in a reduction of primary productivity (Fig. 3). Whether this was simply a dilution effect or whether the rain water itself inhibited primary productivity is unknown. Rainfall effects were minimized as much as possible by subtracting the primary productivity of the control enclosure from that of each of the treatment enclosures. When this was done it became evident that PO_4 additions resulted in significant increases in primary productivity. Nitrate additions did not significantly increase primary productivity in the fall series but were extremely effective in the spring and summer series. In a sense, the fall series was an anomaly in that no algal bloom developed in any of the treated enclosures and primary productivity remained relatively low in all but the phosphate enriched enclosures. Low light intensities and heavy rainfalls (Dickman MS 1968) during the fall series were major contributing factors to the low primary productivity recorded in September and October in both the treated and control enclosures.

The likelihood that the combined $NO_3 + PO_4$ addition would give the highest increase in primary productivity was confirmed in both the spring and summer series. Also, this treatment stimulated the quickest bloom response in these two seasons. Primary productivity in the $NO_3 + PO_4$ treated enclosures was two to three times as high as in any of the other enclosures. As with the nitrate addition

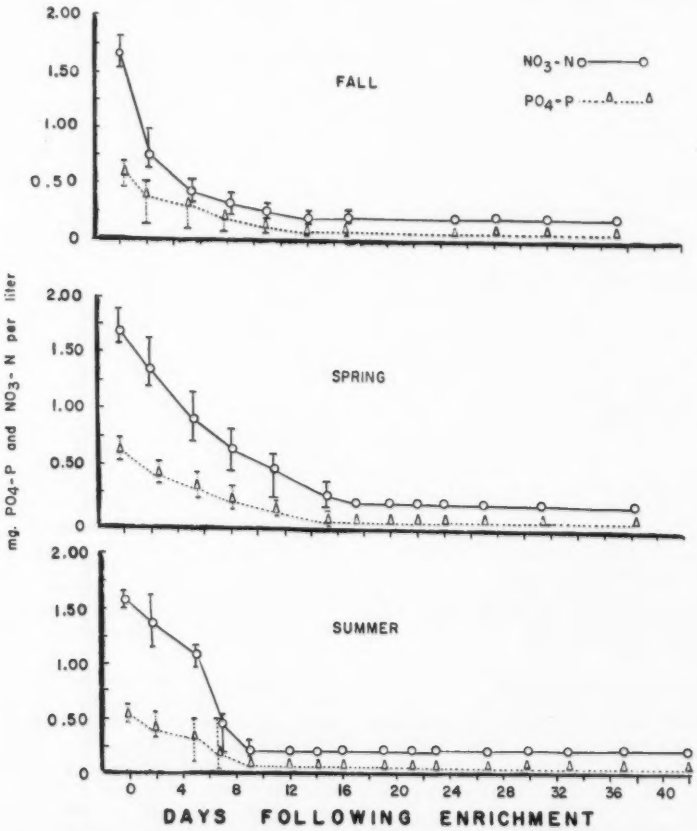

FIG. 2. Total dissolved nitrate and phosphate concentrations in the experimental enclosures following nutrient enrichment in each of three seasons. Vertical lines represent 95% confidence limits about the mean ($n = 8$). The absence of confidence limits indicates that the nutrient concentration was below the minimum value detectable using "Hach" chemicals and techniques.

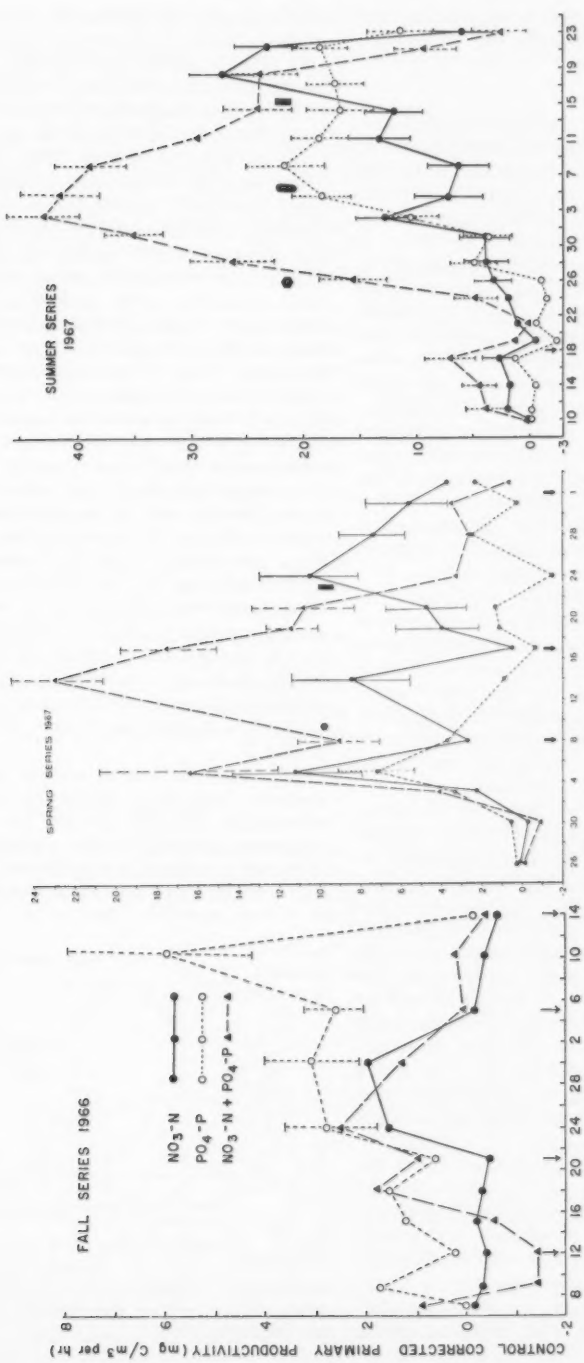

FIG. 3. Primary productivity in the enclosures following enrichment (Sept. 7, 1966; Apr. 26, 1967; July 11, 1967). The primary productivity values of the control (unenriched) enclosures were subtracted from those of the enriched enclosures to give "control corrected" values. Vertical arrows represent days with high rainfall. Days on which algal blooms were first noted are indicated by a rectangle (NO_3), oval (PO_4) and hexagon (NO_3+PO_4); blooms did not occur in all of the enclosures. Vertical lines represent 95% confidence limits and are used only where the mean was significantly different ($\alpha = 0.05$) from the control mean.

in the fall series the nitrate–phosphate addition failed to ellicit a significant response in primary productivity.

Effects on species density — The simplifying concept of a single limiting factor proposed by Schindler (1971) was not supported by this study. In the fall series, only *Microcystis* sp. responded ($P = 0.05$) to phosphate addition while *Synedra filiformis* and *Scenedesmus denticulatus* responded to N + P addition and only *Synedra filiformis* responded to N addition. This same pattern held true in the spring and summer enrichment series. Thus, the species which responded to phosphate additions were entirely different from those responding to nitrate addition. The addition of nitrate and phosphate in combination often stimulated still other species.

During spring, addition of phosphate stimulated an increase in the density of *Cryptomonas erosa*, as did additions of nitrate and N+P. However, this species failed to respond to enrichment in any other season. Likewise, *Ankistrodesmus spiralis* formed dense colonies following nitrate and N+P additions in spring but not in summer or fall.

During summer, *Scenedesmus denticulatus* responded only to nitrate addition while *Coelosphaerium* sp., *Elakatothrix gelatinosa*, *Chlamydomonas* sp., and *Chrysapsis* sp. responded to N + P additions. *Cryptomonas ovata*, on the other hand, responded only to phosphate additions while *Chrysapsis* sp. responded to both P and N + P additions. But, as for *Cryptomonas ovata*, this enrichment response was elicited only in summer.

In addition to the positive responses to enrichment, there were numerous negative ones as well where a species suddenly decreased in density in one or more of the treated enclosures (Table 2). This complex response pattern to enrichment implies that no single nutrient or factor can be expected to limit the growth of all the algal species in a lake through time. Instead, phosphate addition in June stimulated those species which reproduce well at relatively low water temperatures and the other prevailing conditions, such as high light and phosphate levels; and phosphate addition in spring stimulated another group of phosphate-loving species capable of rapid reproduction at the conditions prevailing then.

The addition of nitrate or phosphate did not immediately alter the density of most of the species. One or two of the rarer species, however, generally responded markedly to enrichment by greatly increasing in relative abundance. Of the 285 autotrophic species identified in the study, only 21 (Table 2) significantly increased in relative abundance following nutrient addition. The remaining species increased only slightly if at all. As the concentration of the added nutrient returned to its pre-enrichment level (Fig. 2), different species came to predominate in the enriched enclosures. Concomitantly, many of the species which were abundant prior to enrichment became rare relative to the species which produced the algal blooms (500/ml; Lackey 1945).

The addition of nutrients to each of the enclosures resulted in four basic autotrophic response patterns. (1) A few species (27% of those which gave significant responses) rapidly reached bloom densities. These were always species which were already fairly numerous in the enclosure prior to enrichment. (2) If the species which responded were rare at the time of enrichment (this was usually the case), a longer period of time was needed for them to attain bloom densities (approximately 2 weeks). Nearly half (45%) of all the species which bloomed following enrichment fell into this "lag" response category (Fig. 4). (3) A third group of species (28%) took nearly a month to attain bloom concentrations. It was felt that these species were not responding directly to the initial enrichment but to some secondary substance(s) which may have been a result of it. Thus, this third group of species formed the equivalent of successional seres, which were indicated by plateaus in the community's diversity (Fig. 6). (4) The last group of species decreased significantly in density following enrichment. Of the 28 autotrophic species which gave a significant response to enrichment, 7 fell into this category (Table 2).

This study affords a comparison between the response patterns of particular species and of a community following nutrient addition. After enrichment, productivity of a particular species was inhibited, stimulated, or unaffected, whereas that of the community (Fig. 3) was always stimulated.

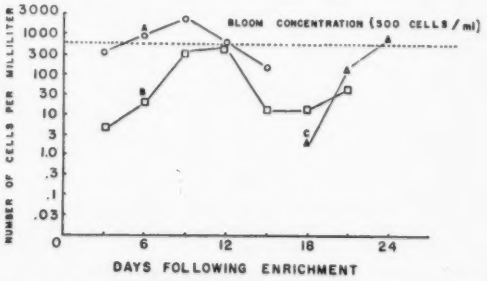

FIG. 4. Examples of three types of positive response to fertilization: rapid (A, *Chlamydomonas* sp., summer series, $NO_3 + PO_4$); lag (B, *Ochromonas* sp., summer series, $NO_3 + PO_4$); and delayed (C, *Trachelomonas* sp., fall series, NO_3).

Conclusions

In general, artificial fertilization did not appear to greatly enhance the primary productivity of the majority of species detected at the time of fertilization. Instead, a few previously rare species rapidly increased until they formed an algal bloom 2–3 weeks after fertilization. This striking increase in density of a few algal species resulted in a sharp reduction in the community's coefficient of diversity (Hp). However, the total number of species in the samples changed very little following enrichment (Fig. 5).

The study indicated that the overall response of the phytoplankton to enrichment is misleadingly simplified as "bloom formation followed by a return to high diversity". Once a bloom was evident, analysis of its species composition indicated that major changes occurred in a few weeks following its formation. Consequently, the species responsible for forming the bloom were often supplanted within a week by a second group of species. This resulted in a succession in which diversity (Hp) increased in spurts (Fig. 6). Superimposed on this was the negative response of the species which were inhibited by the conditions which stimulated bloom formation.

Only by considering the responses of individual species will it be possible to comprehend the biotic phenomena associated with rapid eutrophication. The species specific pattern indicates that bloom formation is only one part of a more complicated picture.

Acknowledgments

We wish to thank Dr P. Larkin, A. Fowler, S. Boyer, and S. Borden for their advice concerning the statistical treatment and computer programming of the data. Drs J. Stein, F. J. R. Taylor, and D. Blinn provided keys and advice concerning taxonomic identification for which we are most grateful. Dr A. Cattell kindly analyzed the water from stations X and Y for nitrate and phosphate

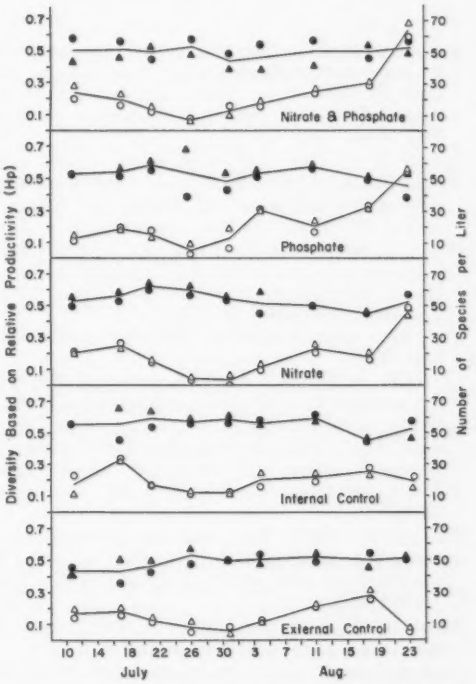

FIG. 5. Species diversity (Hp) and numbers of species observed during the summer enrichment experiments. Solid triangles: numbers of species in north enclosures; solid circles: those in south; open triangles: diversity in north enclosures; open circles: that in south. All but the control enclosures were enriched on July, 11 1967.

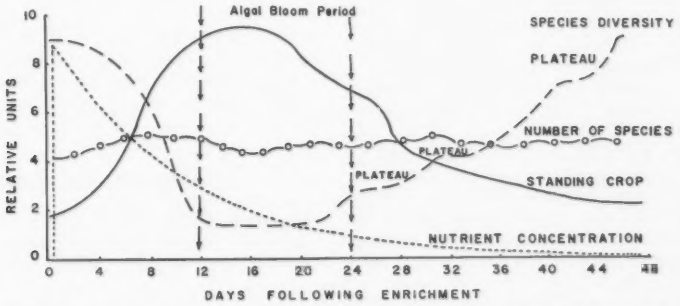

FIG. 6. Generalized response patterns for three factors following artificial enrichment, all three series combined. The retrogressive succession terminated approximately 2.5 weeks after fertilization.

concentrations. We are also indebted to D. Dickman, G. Davies, J. Mathias, and K. Tsumura, who assisted in many ways in the data collection.

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1965. Standard methods for the examination of water and wastewater. 12th ed. New York, N.Y. 769 p.

BROOK, A. J., AND W. B. WOODWARD. 1965. Some observations on the effects of water inflow and outflow on the plankton of small lakes. J. Anim. Ecol. 25: 22–35.

DICKMAN, M. D. MS 1968. The relation of freshwater plankton productivity to species composition during induced successions. Ph.D. Thesis. Univ. of British Columbia, Vancouver, B.C. 115 p.

1968. Some indices of diversity. Ecology 49: 1191–1193.

1969. Some effects of lake renewal on phytoplankton productivity and species composition. Limnol. Oceanogr. 14: 660–666.

EFFORD, I. E. 1967. Temporal and spatial differences in phytoplankton productivity in Marion Lake, British Columbia. J. Fish. Res. Bd. Canada 24: 2283–2307.

EINSELE, W. 1941. Die Umsetzung von zugefuhrtem, anorganischen phosphat in eutrophen see und ihre Ruchwerkung auf seinen Gesamthauskalt. Fisch 39: 407–488.

FISHER, R. A. 1951. The design of experiments. 6th ed. Oliver & Boyd Ltd., Edinburgh. 356 p.

FOURNIER, R. O. 1966. Some implications of nutrient enrichment on different temporal stages of a phytoplankton community. Chesapeake Sci. 7: 11–19.

GOLDMAN, C. R. 1963. The carbon-14 bioassay technique. Symp. Mar. Prod. Pac. Proc. 10th Pac. Sci. Congr. p. 4–22.

HAYES, F. R., J. A. MCCARTER, M. L. CAMERON, AND D. A. LIVINGSTON. 1952. On the kinetics of phosphorus exchange in lakes. J. Ecol. 40: 202–216.

HUTCHINSON, G. E. 1967. A treatise on limnology. Vol. 2. John Wiley & Sons, Inc., New York, N.Y. 115 p.

KUTKUHN, J. H. 1958. Notes on the precision of numerical and volumetric plankton estimates from small sample concentrates. Limnol. Oceanogr. 3: 69–83.

LACKEY, J. B. 1945. Plankton productivity of certain south-eastern Wisconsin lakes as related to fertilization. II. Productivity. Sewage Works J. 17: 795–802.

MARGALEF, R. 1962. Succession in marine populations. Advan. Frontiers Plant Sci. 2: 137–188.

1969. A manual on methods for measuring primary production in aquatic environments, p. 7–14. *In* R. Vollenweider [ed.] I.B.P. Handbook. Number 12. F. A. Davis Co., Philadelphia, Pa.

PHILLIPS, J. E. 1964. The ecological role of phosphorus in waters with special reference to microorganisms, p. 61–81. *In* Hekelekian and Dondero [ed.] Principles and applications in aquatic microbiology. John Wiley & Sons, Inc., New York, N.Y.

PRESCOTT, G. W. 1960. Biological disturbances resulting from algal populations in standing waters, p. 22–37. *In* [ed.] The ecology of algal species. Publ. No. 2. Pymatuning Lab. Field Biol. Univ. of Pittsburg,. Pittsb., Pa.

RUTTNER, F. 1953. Fundamentals of limnology. University of Toronto Press, Toronto, Ont. 242 p.

SCHINDLER, D. W. 1971. Carbon, nitrogen and phosphorus and the eutrophication of freshwater lakes. J. Phycol. 7: 321–329.

SHANNON, E. C. 1948. A mathematical theory of communication. Bell Syst. Tech. J. 27: 379–423 and 623–656.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1960. A manual of sea water analysis. Fish. Res. Board Can. Bull. 125: 1–78.

SWALE, E. M. F. 1964. A study of phytoplankton of a calcareous river. J. Ecol. 52: 433–446.

WILHM, J. L. 1968. Use of biomass units in Shannon's formula. Ecology 49: 153–156.

Voltage and Pulse Rates for Inducing Electrotaxis in Twelve Coastal Pelagic and Bottom Fishes[1]

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KLIMA, E. F. 1972. Voltage and pulse rates for inducing electrotaxis in twelve coastal pelagic and bottom fishes. J. Fish. Res. Bd. Canada 29: 1605–1614.

Laboratory observations on fish reactions to different voltages and direct current pulse rates provided information on the electrical characteristics needed for inducing electrotaxis for use in a proposed automated harvesting system. Optimal electrical combinations for 12 species of coastal pelagic and bottom fishes varied according to species and length, and ranged from 1.5 to 3.0 v/10 cm at 15–45 pulses/sec with a pulse duration of 1.0 msec.

KLIMA, E. F. 1972. Voltage and pulse rates for inducing electrotaxis in twelve coastal pelagic and bottom fishes. J. Fish. Res. Bd. Canada 29: 1605–1614.

Des observations en laboratoire sur la réaction des poissons à différents voltages et à différents taux d'impulsion de cd nous ont renseigné sur les caractéristiques de courant nécessaires à une récolte efficace. Les caractéristiques optimales de courant requises pour déclancher la galvanotaxie chez 12 espèces de poissons pélagiques côtiers et benthiques varient selon l'espèce et la longueur, et s'échelonnent de 1.5 à 3.0 volts/10 cm à une fréquence de 15 à 45 impulsions/sec et une durée d'impulsion de 1.0 msec.

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THE National Marine Fisheries Service Harvesting Technology Program at Pascagoula, Mississippi, has been engaged in the design and development of a harvesting system capable of economically exploiting herring-like fishes in many parts of the world but specifically in the Gulf of Mexico and the Atlantic where these resources are presently unavailable to conventional fishing techniques (Bullis and Carpenter 1968; Klima 1970a). This research has been centered on the development of a mobile automated fishing platform using lights, electrical fields, and underwater rafts to attract fish; and pumps to capture them (Klima 1970a; Klima and Wickham 1971).

This report concerns a laboratory study conducted on the combinations of electrical voltage and pulse rate needed to control and lead coastal pelagic and bottom fishes found in the eastern Gulf of Mexico and tropical Atlantic.

Materials and Methods

Schooling coastal pelagic species tested were chub mackerel (*Scomber japonicus*), scaled sardine (*Harengula pensacolae*), Spanish sardine (*Sardinella anchovia*), round scad (*Decapterus punctatus*), bumper (*Chloroscombrus chrysurus*), rough scad (*Trachurus lathami*), Atlantic thread herring (*Opisthonema oglinum*), round herring (*Etrumeus teres*), silver anchovy (*Engraulis eurystole*), and butterfish (*Peprilus triacanthus*). Bottom fishes tested were Atlantic croaker (*Micropogon undulatus*), spot (*Leiostomus xanthurus*), and longspine porgy (*Stenotomus caprinus*).

Bottomfish and the coastal pelagic butterfish were caught with trawls off the Mississippi Coast. The other coastal pelagic fishes were caught at night with lights and a 5-m lift net in the northern Gulf of Mexico (Wickham 1970). The fish were held in tanks of circulating sea water before being transported to the Laboratory. Only fish in good physical condition were used. Electrical studies were conducted on 2885 fish, each subjected to only one test and then discarded.

The studies were done in an insulated tank 4.5 × 1.2 m and 1 m high with 2700 liters of water ranging in salinity from 20.0 to 29.0‰ and at a temperature of 15–22 C.

General Electric and NMFS staff engineers modified our electric shrimp pulse generator (Klima et al. 1969) for this study. The modified generator converts alternating current to pulsed direct current in the form of capacitor discharge pulses at a rate of 5–75/sec as measured with an electronic counter. Pulses were applied to two monel metal electrodes, 1.08 m square × 3 mm thick, mounted at opposite ends of a tank. The system provides a relatively uniform field throughout the experimental tank except at the side edges of the electrode. Pulse characteristics were measured by means of a pair

[1]Contribution No. 235.

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of pickup probes made from two 3 mm diameter bronze rods spaced 10 cm apart and insulated so that only the bottom 10 mm of each rod was exposed. The pulse characteristics were displayed on the oscilloscope as a graph of voltage against time. Voltage is referred to as the decrease across 10 cm.

The wave form on the oscilloscope is not a true capacitor discharge-shaped pulse because of the inductance of the external circuit. For this reason rise time was disregarded and I considered the peak of the pulse as time zero. The remaining portion of the pulse closely approximates a theoretical capacitor discharge shape.

Pulse width is defined here as the time in microseconds (μsec) it takes a pulse to decay to one-third of its peak value, as is standard in describing capacitor discharges. Values for this parameter ranged from 0.5 to 2.2 msec in the study. These pulse widths were found most effective for electrical stimulation.

Water resistance, used to calculate the theoretical power requirements of the prototype electrical fishing system, was measured by placing a 1 ohm resistor on the output side between the pulse generator and one electrode. The amperage in the resistor at a specific d-c voltage output was measured via pickup leads from the oscilloscope. The total voltage from electrode to electrode was divided by the amperes in the resistor in order to obtain the resistance between the electrodes. For example, 2.0 v per 10 cm (v/10 cm) in the tank gives 90 v across the tank (i.e. $2.0 \times 10 \times 4.5$ m = 90 v) which, divided by 38 amp through the resistor, gives 2.4 ohms resistance in the tank.

To determine the combinations of electric parameters which best induced electrotaxis, I positioned the fish facing, and within 1 m of, the cathode. Preselected pulse rates and voltages were applied to the experimental tank only when the fish were correctly positioned. Two event-timing clocks connected to the pulse generator were activated at the same time as the pulser to provide a time base for measuring responses. The observer could stop each clock when the animal had completed a specific reaction.

To evaluate the effectiveness of the pulse characteristics tested, I measured the time the fish took to swim the length of the tank. In addition, the proportion which went into tetanus was recorded. I selected one lap to record tetanus because any fish will eventually go into tetanus if subjected to electricity for a long period of even low voltage (Collins et al. 1954).

In another series of tests to further evaluate pulse characteristics, I forced the fish to swim up and down the tank, and these are subsequently referred to as leading studies. Leading was accomplished by changing the polarity of the field after each lap, or 4.5 m, when the fish was within 0.5 m of the anode. The process was continued until the fish could no longer swim or until it had swum 20 laps. The times it took to swim two and five laps, and the number of laps it swam, were recorded.

Even with all this information available for certain species, it was difficult to determine the best combination of electric parameters inducing electrotaxis. Consequently, an index of optimal responses was obtained for selected species by dividing the time it took an individual to swim five laps (23 m) by the number of laps each fish had swum and then computing the average for the electrical combination. The lower the index, the better the electrical combination. For example, if a given electrical combination showed a time of 30 sec to swim five laps and a distance swum of 15 laps, the index was 2.

The basic idea behind the experimental design was that fish stimulated with the optimum pulse characteristics would swim further and faster. I considered the shortest times to swim one, two, and five laps; and the average maximum number of laps swum as the most suitable.

Selection of Test Voltages and Pulse Widths

The success of electrical fishing is dependent upon the proper electrical combinations for inducing taxis. The voltage, pulse rate, and pulse width required to induce electrotaxis vary with species, fish size, and, probably, other factors (Riedel 1952; Collins et al. 1954; Bary 1956; Higman 1956; Halsband 1967; Monan and Engstrom 1963; Kessler 1965; Klima 1968). Hence, a combination of electrical factors which will induce electrotaxis in one species may induce either fright response or no response in another.

The overall effect of increasing voltage at a fixed pulse rate was examined for the scaled sardines and these results provided the general guideline for the experimental minimum and maximum test voltages.

A gradual increase was evident in the time it took scaled sardine to swim one lap as the voltage was increased from 1.5 to 4.5 v/10 cm at 15 pulses/sec. The greatest effect was the decrease in the distance swum with an increase in voltage (Fig. 1). The maximum number of laps scaled sardine swam was at 1.5 v/10 cm, and the lowest at 4.5 v. The response times to swim two laps showed a general increase with an increase in voltage.

The overall effect of a voltage increase was to decrease the distance over which scaled sardine could be controlled and to decrease the speed it took to swim more than 9 m. An increase in voltage also increased the proportion of fish in tetanus even at low repetition rates.

Response of fish in an electrical field depends upon voltage and the length of the animal because voltage potential increases proportionately with the animal's length. For example, a 10 cm fish, faced parallel to the current lines in a homogenous field at 30 v/m, has a potential of 3 v from head to tail but a 20 cm fish has a potential of 6 v. As a result, it is important to determine the significance of size or length as related to the most suitable electrical combination. Consequently, one coastal pelagic fish, scaled sardine, and one bottomfish, longspine porgy, of different size groups were examined. Results on scaled sardines indicated that small fish could be led more effectively at 3 v/10 cm than large fish but the responses indicated that 3 v/10 cm are not completely adequate to control these smaller fish (Table 1). Stimulation of less than 3 v/10 cm or more than 1.5 v/10 cm would probably be more suitable.

A comparison of the response times to swim one lap was made between small (86–101 mm fork length) and

large (125–151 mm fork length) longspine porgies. Analysis of the average values by Student's Test did not show a difference between the response times.[1] These results show that the electrotaxis response is independent of size in 86–151 mm longspine porgy for the test conditions (i.e. 1.5 v/10 cm and pulse rates of 30 and 35/sec).

These results dictated care in selection of size groups for this study. Consequently, the results of the most suitable electrical combinations are for the size groups of fish tested.

[1] $t = 1.158$ and 18 df and $t = 0.630$ and 29 df for 30 and 35 pulses/sec, respectively.

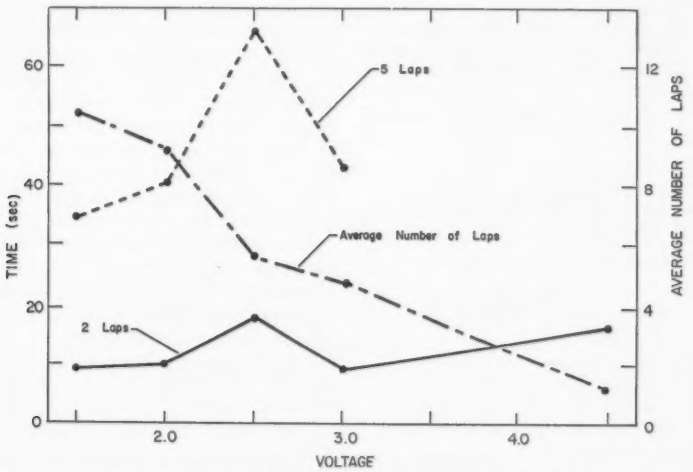

FIG. 1. Times to swim two and five laps and average numbers of laps swum by scaled sardines at different voltages per 10 cm at 15 pulses/sec. Each plotted value is for an initial lot of 7–22 fish.

TABLE 1. Comparison of small (71–95 *mm* fork length) and large (100–145 *mm*) scaled sardine in their ability to be led two and five laps with 1.5 and 3.0 v/10 cm at three pulse rates per second.

	Pulse rate					
	25		35		45	
	small	large	small	large	small	large
			Voltage 1.5			
Avg. no. of laps	5.9	9.0	5.0	9.7	4.2	9.8
Time to swim						
2 laps (*sec*)	25.8	8.2	29.6	6.5	30.3	5.9
5 laps (*sec*)	80.4	30.4	94.0	29.3	102.0	29.2
Optimal						
response index	14.5±3.17[a]	4.1±0.96[a]	18.7±2.26[a]	3.4±1.32[a]	18.7±2.78[a]	3.4±1.1
			Voltage 3.0			
Avg. no. of laps	4.9	2.3	4.6	1.1	3.5	0.9
Time to swim						
2 laps (*sec*)	17.7	10.1	13.9	24.5	19.6	18.6
5 laps (*sec*)	37.5	0	51.8	0	60.0	0
Optimal						
response index	6.2±2.41	infinite	5.4±4.73	infinite	16.8±6.58	infinite

[a]Average and 95% confidence interval (20 fish per group).

In addition to voltage, pulse width affects the responses of fish (Bary 1956). To determine the range of pulse widths most desirable for the experiments, a series of tests were conducted on spot to determine the times to swim one lap at 1.5 v/10 cm with 25 and 30 pulses/sec at pulse widths of 0.7, 1.2, and 2.0 msec (Table 2). At a pulse rate of 25/sec, times ranged from 3.3 to 4.6 sec. At 35 pulses/sec, however, it took an average of 5.4 sec to swim one lap at a pulse width of 0.7 msec, and 3.2 sec at a 1.2 msec pulse width. These differences were tested by analysis of variance, which clearly showed no difference in response times at 35 pulses/sec ($F = 2.26 < F_{.95\ (2,27)} = 3.36$). Because of the closeness in response times at 25 pulses, no analysis of variance was needed.

TABLE 2. Times (*sec*) to swim one lap (4.5 *m*) at three pulse widths for spot stimulated with 1.5 v/10 cm at two pulse rates.

Pulse width (*msec*)	Pulse rate	
	25	35
0.7	4.6±1.11[a,b]	5.4±2.09
1.2	4.6±1.22	3.2±0.88
2.0	3.3±0.41	4.4±1.74[c]

[a]Average and 95% confidence interval.
[b]Thirty fish tested; 10 in other tests.
[c]Nine fish responded.

The responses of fish subject to pulse widths ranging from 0.7 to 2.0 msec were similar; hence, the test animals were subjected to pulse widths within this range.

Effective Combinations

The combinations of electrical parameters which are considered most desirable for inducing electrotaxis and for leading 12 of the 13 species are summarized in Table 3. Little difference existed between the electrical parameters for coastal pelagic and bottom fish, except for croakers, as control was not obtained for this species. However, response times to swim one and two laps and average distance covered differed greatly between the two groups as bottom fish swam at much slower speeds and could not be led as far as the coastal pelagics.

The optimal electrical parameters were similar for longspine porgy and spot; the only difference was that spot could be effectively controlled at a slightly wider range in repetition rate. Croaker could not be effectively controlled (Table 4).

Longspine porgy reaction time curves to swim one lap were similar in shape but not in amplitude for 1.5, 3.0, and 4.5 v/10 cm (Fig. 2). The similarity in shape indicates that voltage has little effect. A decrease in pulse rate increased the time to swim to the anode. Combinations of high voltage and high pulse rates were undesirable for inducing electrotaxis in both longspine porgy and spot.

TABLE 3. Effective combinations of voltage (per 10 *cm*) and pulse rate (per *sec*) for inducing electrotaxis in the species studied and the approximate amperes (per m^2).

Species	(*v*)	Pulses	(*Amp*)	Fish length (*mm*)	
				Range	Avg.
Coastal pelagics					
Scaled sardine	1.5	15–55	62.5	100–145	118
	3.0	8–28	86.5	100–145	118
Spanish sardine	1.5	35–45	43.3	85–180	130
	3.0	15	86.5	85–180	130
Round herring	3.0	25–45	86.5	80–150	104
Silver anchovy	3.0	35–45	98.3	85–110	96
Butterfish	1.5	35	41.3	80–100	118
	3.0	45	86.5	80–160	118
Chub mackerel	1.5	15	53.8	125–240	180
Bumper	1.5	15	57.0	135–230	173
Rough scad	1.5	15–25	42.3	120–145	133
Thread herring	1.5	15	62.5	80–185	146
Round scad	1.5	15	43.3	90–170	148
Bottom fish					
Spot	1.5	15–35	43.3	90–250	120
Longspine porgy	1.5	25–35	43.3	100–160	128

TABLE 4. Average times in seconds to swim one lap (4.5 *m*) at various voltages and pulse rates for three fishes.

Volts per 10 cm	Pulses per sec					
	5	15	25	35	45	55
			Butterfish			
1.0	9.7±1.96[a]	7.8±2.36	12.0±4.58	4.4±1.20	4.7±1.02	4.9±0.90
	10(10)[b]	10(10)	10(10)	11(11)	15(15)	8(10;5)[c]
3.0	16.3±4.85	7.3±3.44	4.5±1.22	7.0±2.46	1.7±0.45	5.1±1.35
	9(10)	10(10)	10(10)	10(10)	10(10)	7(10)
4.5				7.9±2.09		
				4(6;6)		
			Croaker			
1.5	34.4±11.03	8.9	0	25.0±15.37	0	0
	5(10)	1(10)	0(11;10)	2(10;8)	0(10;10)	0(3;3)
3.0	19.7±14.59	6.8±3.11	0	0		
	10(10)	6(10;6)	0(11;11)	0(10;10)		
4.5	13.1±3.88	0	0	0		
	6(12)	0(10;10)	0(10;10)	0(10;10)		
			Chub mackerel			
1.5		3.6±1.58	5.7±1.90	8.3±4.47	6.1±6.83	
		6(6)	10(10)	7(10;6)	10(10;1)	
3.0			4.9±3.50			
			4(4)			

[a]Average and 95% confidence interval.
[b]Number of fish responding and number tested.
[c]The second numeral in parentheses is the number in tetanus.

The effective pulse rate for coastal pelagics varied widely for all but four of the ten coastal pelagic fishes (Table 3). The four had specific repetition rate requirements of 15 pulses/sec. There was no relation between the type of fish and optimal pulse rate; however, a low repetition rate is much more effective for developing the proper electrical field in terms of power requirements.

Included in Table 3 is a list of current densities. These were calculated for sea water from resistances of 1.8 to about 2.6 ohms, depending on salinity and temperature, low salinity and temperature giving a greater resistance. Using the calculated values of resistance, the current was computed (i.e. $I = \frac{E}{R}$ (Sears and Semansky 1955)) at 26.0–37.5 amp at 15 v/m (i.e. total voltage = 67.5). The current density, $J = \frac{I}{A}$, where A is the area, ranged from 43.3 to 62.5 amp/m^2. At 3.0 v/10 cm the current density was twice as large as at 1.5 v/10 cm. In terms of the most efficient power requirements the lower potential is better than the higher.

Spanish sardine, scaled sardine, and butterfish were the only species which had two effective voltages: 1.5 and 3.0 v/10 cm. As expected, the higher voltage had twice the current density.

Considerable differences were exhibited in the reaction times to swim one lap and percent in tetanus at different voltages for both scaled sardine and Spanish sardine (Fig. 3). Percent of fish in tetanus was greatest at the highest voltage and decreased with a decrease in voltage. At the intermediate and highest voltages, the percent of fish in tetanus increased with pulse rate. The shortest times for one lap were from 15 to 55 pulses/sec at 1.5 v/10 cm for scaled sardine and from 15 to 25 pulses/sec at 3 v/10 cm for Spanish sardines. The indexes of optimal response indicate the combinations of 1.5 v/10 cm at 35 and 45 pulses/sec and 3 v/10 cm at 15 pulses/sec to be the most effective for Spanish sardine, and 1.5 v/10 cm at 15–55 pulses/sec for scaled sardine.

The most effective electrical combinations for round herring (Fig. 4) and silver anchovy (Fig. 2) were 3 v/10 cm at 25–45 pulses/sec. The greater potential required by these species is probably due to size. At an average size of about 100 mm total length the voltage drop between head and tail is less than that for longer fish. All of the other tested

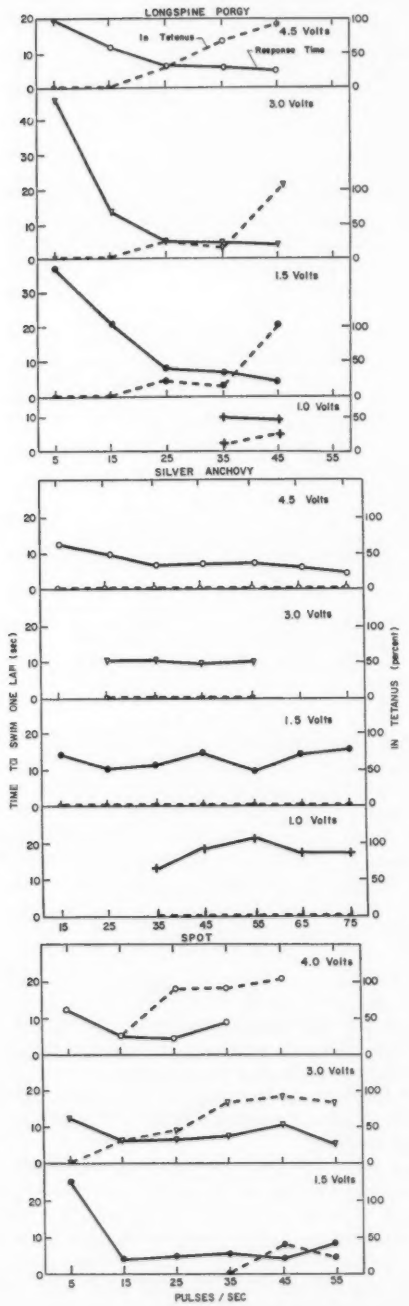

FIG. 2. Times to swim one lap (solid lines), percent in tetanus (broken lines) at end of one lap, and optimal response indexes at various pulse rates and voltages for longspine porgy, silver anchovy, and spot. Each plotted value is for an initial lot of 10–31 fish.

species were on the average longer than the round herring and silver anchovy. I was unable to capture specimens of a larger size to confirm the above hypothesis. Presumably, larger round herring and silver anchovy would also require only 1.5 v/10 cm stimulation to induce electrotaxis.

Inability to capture adequate quantities of live fish limited the experimental tests on the remaining species. The most suitable combinations to induce electrotaxis appeared to be 1.5 v/10 cm at 15 pulses/sec for chub mackerel (Table 4), bumper (Fig. 5), and thread herring (Table 5); and 1.5 v/10 cm at 15–25 pulses/sec for round scad (Table 5) and rough scad (Fig. 6).

Fishing Applications

Fishing systems which attract and concentrate fish could use electricity to lead fish to the anode for economical extraction with pumps. Preliminary information is provided on an electrode configuration and its power requirements. This electrode type, when used with lights and pumps, should be suitable for commercial harvesting of coastal pelagic fishes in many areas of the world.

Guiding or leading coastal pelagic and bottom fish to the anode is most easily accomplished within a uniform electric field. Nonuniform fields, as the name implies, have a gradation of voltage which increases as the fish approach the anode. Thus, fish at the outer edge of a nonuniform electric field undergo a fright reaction because of the low voltage gradient. Fish in the center of the field undergo electrotaxis and swim toward the anode until the voltage drop is sufficient to cause tetanus. Fright and tetanus responses are undesirable for leading fish. In a uniform field, however, fish can be led considerable distances to the anode without a tetanus reaction. An ideal fishing situation can be established, therefore, with a uniform electric field combining the proper parameters.

The results of this study have been used to calculate the power requirements for field electrodes for a harvesting system. The parameters used were 15–30 v/m at pulse rates ranging from 15 to 45, with a capacitor discharge pulse shape at a pulse width of 1.0 msec. It was assumed that this system would operate in sea water with a salinity of 30‰, a temperature of 25 C, and a resistivity of 0.213 ohm-meters (Myers et al. 1969). This configuration should provide approximate uniform fields between

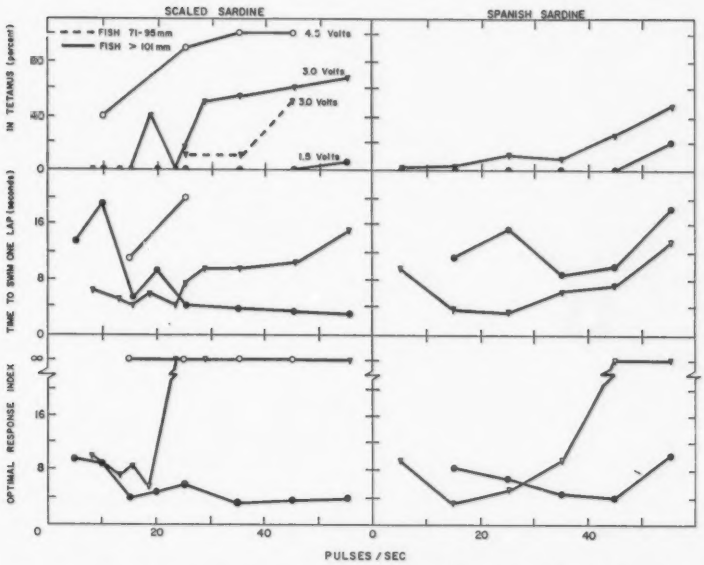

FIG. 3. Times to swim one lap, percent in tetanus at end of one lap, and optimal response indexes at various pulse rates and voltages for scaled sardine and Spanish sardine. Each plotted value is for an initial lot of 7–22 fish.

the electrodes ranging from 15 to 30 v/m. The gradient would decrease slowly to the edge of the plates, where it would drop off more quickly, but the gradient near the edges of this plate would be slightly higher. The electric field in this configuration varies as follows:

For parallel plates separated by a distance D

$$F = \frac{E}{D} \text{ v/m} \qquad (1)$$

where F is the v/m in the field, E is applied voltage. The resistance of sea water between the electrodes is:

$$R = \frac{\rho \times D}{A} \qquad (2)$$

where ρ is the resistivity of sea water in ohm-meters, and A is the surface area of electrodes; however, this formula only describes the resistance of the volume of water between the electrodes. Resistance measurements from empirical field measurements with parallel plates (when field fringing was considered) are best described by a value five times less than calculations by Formula (2) (Klima 1970b; W. Seidel and C. Kreutzer personal communication).

In comparing the electrode configuration, I assumed the dimensions consisted of two 10 × 10 m plates separated by 20 m; therefore,

$$R = \frac{\rho \times D}{A \times 5} = \frac{0.213 \times 20}{100 \times 5} = 0.0085 \text{ ohms;} \qquad (3)$$

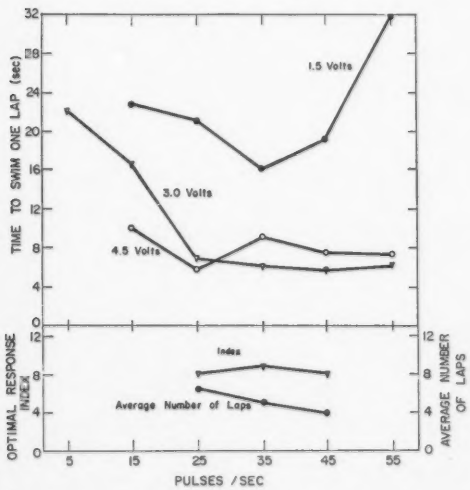

FIG. 4. Times to swim one lap and optimal response indexes for round herring at various pulse rates and voltages. Each plotted value is for an initial lot of 10–15 fish.

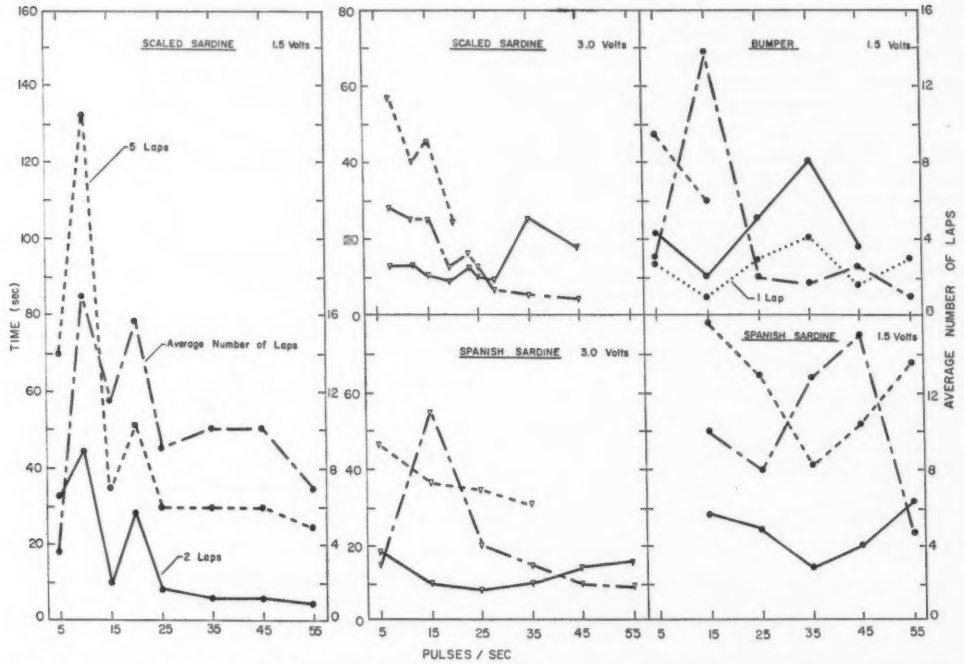

FIG. 5. Times to swim two and five laps and average numbers of laps swum for scaled sardine, Spanish sardine, and bumper at various pulse rates and voltages. Each plotted value is for an initial lot of 6–22 fish.

the applied voltage,

$$E = FD = 15 \times 20 = 300 \text{ v}; \quad (4)$$

and the current,

$$I = \frac{E}{R} = \frac{300}{0.426} = 35{,}294 \text{ amp.} \quad (5)$$

The disadvantages of this system are: (1) the electrical field is not confined, making the effects of fringing on the electrical field and its interference with a vessel or platform more pronounced; (2) the system requires relatively high voltage causing a safety hazard to operating personnel; and (3) the fish are directed to a relatively large area making capture difficult.

These data show that the parallel plate can provide an effective electrode configuration from the standpoint of power requirements and an approximate uniform electrical field. Smaller size electrodes are suggested to eliminate the problem of capturing fish from a large anode (i.e. 10 × 10 plate). Rather than one anode and one cathode, two cathodes (rectangular plates or grids) can be positioned about 10 m to the front and back faces of the anode. The anode consists of two rectangular grids with an open space between where a suction pump for extracting fish can be placed. The sides and bottom of the anodes can be fitted with material to prevent fish from escaping, or a small secondary electrical field can be used in conjunction with the pump, similar to Kreutzer's electrical fish pump system (Kreutzer 1964).

The uniform field between the cathode and anode forces the fish to swim into the anode grid. Since the primary field between the anodes does not exist, the fish should be able to stay within this area until either the current is turned off or they are captured by the secondary field and its pump. Fish are prevented from swimming out of the area between the anodes by the primary electrical field. The space between the anodes may be increased or decreased without electrical power changes.

In the above system, assuming for each cathode a surface area of 50 m^2 generating a uniform field of 15 v/m requires a potential of 300 v and a current pulse of 17,647 amp. A peak power pulse of 5,294 kw is generated. Using a 15 pulse/sec repetition rate, at a pulse duration of 1.0 msec, the average power

TABLE 5. Summary of leading studies with round scad and thread herring at 1.5 v/10 cm and two pulse rates.

Pulses per sec	Time (*sec*) 2 laps	Time (*sec*) 5 laps	Avg. no. of laps	Optimal response index
		Round scad[a]		
15	13.2±4.00[b]	45.1±3.46	12.2±3.29	3.7±0.97
25	14.8±7.32	44.3(3)[c]	5.5±3.70	6.5±14.07(3)
		Thread herring		
15[d]	23.8±10.15[10]	59.8±23.97	13.2±6.17	5.2±2.78
25	17.1±4.50(9)	38.5±27.61(2)	3.6±1.18	6.4±4.21(2)

[a]Ten fish in each test.
[b]Average and 95% confidence interval.
[c]Number of fish that responded.
[d]Five fish in these tests and 10 in others.

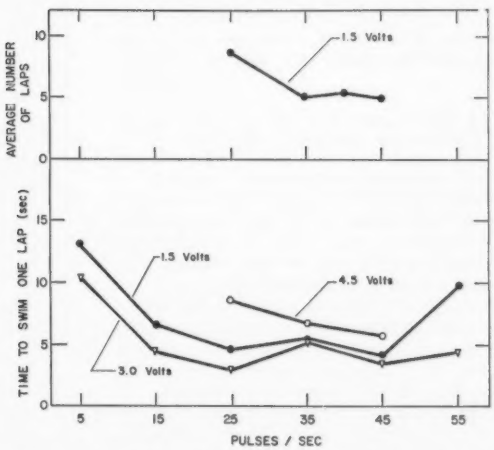

FIG. 6. Times to swim one lap and average numbers of laps swum at various pulse rates and voltages for rough scad. Each plotted value is for an initial lot of 5–10 fish.

required is about 79.4 kw. An increase in pulse rate to 25/sec would require 132.1 kw for 15 v/m.

This proposed fishing system could be developed with off-the-shelf components now available (G. McGee personal communication; C. Kreutzer personal communication).

Acknowledgments

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I would also like to thank Messrs G. McGee and R. Emerson of General Electric Corporation, Bay St. Louis, Mississippi, for their engineering advice and for updating of the pulse generator for this study. Messrs D. A. Wickham, G. M. Russell, and R. S. Ford, Jr. assisted in the collection of live specimens. I would further like to acknowledge the assistance of Messrs W. Seidel and F. J. Hightower who were able to maintain electrical equipment in working condition.

BARY, B. M. 1956. The effect of electric fields on marine fishes. N.Z. Def. Sci. Corps Nat. Inst. Oceanogr. Wormley M. Godalming Surv. Her Majesty's Stationery Off. Edinburgh. 32 p.

BULLIS, H. R., JR., AND J. S. CARPENTER. 1968. Latent fishery resources of the central and Atlantic region. The future of the fishing industry of the United States. Univ. Wash. Publ. Fish. N. S. 4: 61–64.

COLLINS, G. B., C. D. VOLZ, AND P. S. TREFETHEN. 1954. Mortality of salmon fingerlings exposed to pulsating direct current. U.S. Fish Wildl. Serv. Bur. Commer. Fish. Fish. Bull. 92: 61–81.

HALSBAND, E. 1967. Basic principles of electric fishing, p. 57–64. *In* Fishing with electricity—its applications to biology and management. Fishing News (Books) Ltd., London.

HIGMAN, J. B. 1956. The behavior of pink grooved shrimp, *Penaeus duorarum*, Burkenroad, in a direct current electrical field. Fla. State Bd. Conserv. Tech. Ser. 16: 1–23.

KESSLER, D. W. 1965. Electrical threshold responses of pink shrimp, *Penaeus duorarum*, Burkenroad. Bull. Mar. Sci. 15: 885–895.

KLIMA, E. F. 1968. Shrimp-behavior studies underlying the development of the electric shrimp-trawl

system. U.S. Fish Wildl. Serv. Bur. Commer. Fish. Fish. Ind. Res. 4: 165–181.

1970a. Development of an advanced high seas fishery and processing system. Mar. Technol. Soc. J. 4(5): 80–87.

1970b. R/V George M. Bowers cruise report, 1 p. U.S. Dep. Inter. Bur. Commer. Fish.

KLIMA, E. F., W. R. SEIDEL, W. DEGROVE, AND F. J. HIGHTOWER. 1969. Electrical trawl net system. U.S. Pat. Off. U.S. Pat. No. 3,483,649.

KLIMA, E. F., AND D. A. WICKHAM. 1971. Attraction of coastal pelagic fishes with artificial structures. Trans. Amer. Fish. Soc. 100: 86–99.

KREUTZER, C. O. 1964. Utilization of fish reactions to electricity in sea fishing. Mod. Fish. Gear World 2: 581–582.

MONAN, G. E., AND D. E. ENGSTROM. 1963. Development of a mathematical relationship between electric-field parameters and the electrical characteristics of fish. U.S. Fish Wildl. Serv. Fish. Bull. 63: 123–136.

MYERS, J. J., C. H. HOLM, AND R. F. MCALLISTER. 1969. Handbook of ocean and underwater engineering. McGraw-Hill Book Company, Inc., New York, N.Y. 112 p.

RIEDEL, D. 1952. Concerning the influence of electric current on the sexual products of fish with special emphasis on electro-fishing. Inst. Fisch. Dtsch. Akad. Landwirtsch.-Wiss. Berlin. 53 p.

SEARS, F. W., AND M. W. SEMANSKY. 1955. Electricity and magnetism optics, and atomic physics, p. 403–973. *In* University physics. 2nd ed. Addison-Wesley Publishing Company, Inc., Reading, Mass.

WICKHAM, D. A. 1970. Methods and equipment for collecting live coastal pelagic fishes with artificial light and a 5-meter lift net. Commer. Fish. Rev. 32(12): 52–57.

Food Specialization By Individual Trout

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BRYAN, J. E., AND P. A. LARKIN. 1972. Food specialization by individual trout. J. Fish. Res. Bd. Canada 29: 1615–1624.

Analyses of stomach contents showed that the kinds of prey eaten by brook trout (*Salvelinus fontinalis*), cutthroat trout (*Salmo clarki*), and rainbow trout (*Salmo gairdneri*) were seldom distributed at random among the individuals. Repeated observation of food eaten by individuals in a stream and ponds showed that prey types were eaten in proportions which were characteristic for an individual.

Specialization occurred on several different kinds of prey. Although the degree of specialization was higher during shorter intervals, the data suggested that some specialization persisted for half a year. There were no striking correlations between degree of specialization and other individual properties such as size, growth rate, weight of food, number of food items, previous specialization, or area of recapture.

In addition to the observations on trout in relatively undisturbed habitats, a field experiment was conducted using laboratory-reared rainbow trout held in small ponds. The food of each trout in the experiment was sampled repeatedly. In analysis of variance, interaction among the individuals and kinds of prey eaten showed that food specialization occurred. Both the absolute and relative abundance of potential prey were constant during the experiment.

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L'analyse des contenus stomacaux révèle que les proies avalées par l'omble de fontaine (*Salvelinus fontinalis*), la truite fardée (*Salmo clarki*), et la truite arc-en-ciel (*Salmo gairdneri*) sont rarement réparties au hasard chez les individus. Des observations répétées sur des poissons habitant un cours d'eau ou gardés dans des étangs démontrent que les types de proies sont mangés dans des proportions caractéristiques d'un individu.

Il y a spécialisation par rapport à plusieurs types de proies. Bien qu'elle soit plus prononcée sur de courtes périodes, cette spécialisation semble persister pendant six mois. Il n'y a pas de corrélation évidente entre le degré de spécialisation et d'autres caractères individuels tels que la taille, le rythme de croissance, le poids de la nourriture, la diversité des types d'aliments, une spécialisation préalable, et l'endroit de recapture.

En plus d'observations sur des truites en milieux relativement non dérangés, nous avons poursuivi une expérience sur le terrain, utilisant des truites arc-en-ciel élevées au laboratoire et gardées dans de petits étangs. Nous avons prélevé sur ces truites des échantillons de nourriture à plusieurs reprises. L'interaction entre les individus et les types de proies avalées, soumise à l'analyse de la variance, démontre qu'il y a spécialisation alimentaire. L'abondance absolue et relative des proies disponibles est demeurée constante durant toute l'expérience.

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IT is a commonplace observation that stomach contents of different individual fish of one species, similar in size and captured at the same time and place, may contain different kinds of food. The question immediately raised is, "Do some individuals repeatedly eat more of some kinds of food than other individuals and, if so, how long does this difference persist?" The analysis of stomach content data usually generates the suspicion that individual fish do tend to have what appear to be different searching techniques or different "tastes," but the evidence is characteristically circumstantial, and there are no data on the food of the same individuals at different times that would more clearly demonstrate these phenomena. The present study is concerned with repeated sampling of the stomach contents of brook trout (*Salvelinus fon-*

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tinalis), cutthroat trout (*Salmo clarki*), and rainbow trout (*Salmo gairdneri*) in a small stream and in a pond; and a series of experimental observations on repeated feeding by rainbow trout in small ponds.

Materials and Methods

Trout were collected with electroshocking apparatus at midday from Marshall Creek, a small, spring-fed stream near Abbotsford, British Columbia (Fig. 1). An additional small group of rainbow trout was sampled from Fishing Pond, tributary to Marshall Creek, three times in March, 1969. Part of the creek (subsections 1A and B) was on the grounds of the Fraser Valley Trout Hatchery and water flowed from the hatchery ponds into the creek. About one-half of the trout in the stream apparently originated from the hatchery. Not all sections of the stream were sampled for the same length of time. Subsection 2B was sampled from September 4, 1968, to get more trout; subsections 3A and B were sampled from March 29, 1969; and 1A and B from April 10, 1969. Subsection 2A was sampled occasionally for experimental animals. All sampling terminated October 9, 1969. The trout were usually sampled every 2 weeks. With a shorter interval the percentage of recaptured trout was lower and the percentage without food was greater.

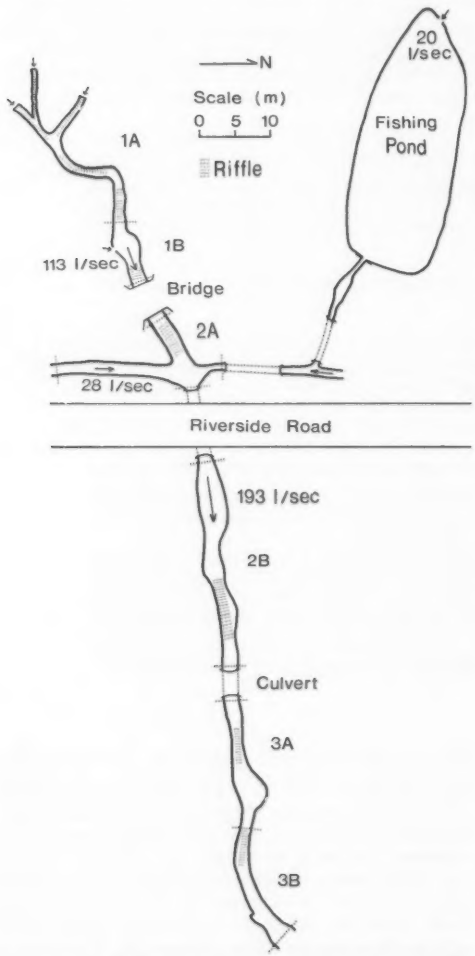

FIG. 1. Portion of Marshall Creek studied and Fishing Pond. The dotted lines indicate subsection boundaries.

Fish were identified by green plastic tags (3 × 10 × 0.5 mm) attached with monofilament just anterior to the dorfal fin. Stomach contents were "flushed out," as described by Seaburg (1957), after the fish were anesthetized with 1-phenoxyethanol at 0.5 ml/liter (Bell 1964). It was assumed that the stomach contents represented nearly all the food eaten 6–8 hr before capture, and from 20 to 80% of that eaten 9–24 hr before capture (Windell and Norris 1969). Of all trout captured or recaptured, 82% had food in the stomach.

Several observations suggested that mortality of trout was high or that trout left the study area. Frequent sampling and the attendant handling were perhaps contributing factors. Most trout were captured only once and none more than six times (Table 1). When one subsection was sampled five times in 3 days, the number of trout captured decreased from eight to three; when three subsections were sampled three times on alternate days, the decrease was from 15 to 1. Of 235 tagged fish, anglers caught six in the study area, eight less than 1 km away, and six more than 1 km away.

Results

Statistical Analysis of Repeated Stomach Samples

Food distribution among individual trout caught at virtually the same time and place was usually heterogeneous, the stomachs of different individuals containing differing proportions of various food items, as shown below from the stomach contents of a representative sample of seven rainbow trout captured in Fishing Pond, March 2, 1969. Kinds

TABLE 1. Frequency of capture of individual trout used for stomach content sampling in Marshall Creek between September 4, 1968, and October 9, 1969.

Maximum interval between capture and recapture	No. of times an individual was captured					
	1	2	3	4	5	6
21 days	167	41	12	5	2	0
250 days	167	54	16	8	6	4

of food for which only one item occurred in one stomach were omitted. Oligochaetes were probably not eaten one at a time. For the whole array of data $\chi^2 = 335.3$; 35 df; $P < .001$ (length given in millimeters under sex symbol):

Food	♂ 440	♀ 381	♀ 458	♀ 443	♂ 563	♀ 413	♀ 490
Corixid	0	2	5	0	0	3	0
Mayfly naiad	0	3	11	2	0	1	0
Damselfly naiad	0	5	3	0	1	5	0
Isopod	0	1	2	0	0	21	0
Amphipod	0	0	5	0	1	3	0
Caddisfly larva	0	0	3	0	0	5	0
Salamander	0	0	0	0	0	0	0
Oligochaete	0	6	33	56	0	42	0

With repeated samples from a series of fish, the hypothesis was tested: the similarity in the stomach contents of the same fish taken at different times is equal to the similarity in the stomach contents of different fish taken at different times. The prey items were categorized according to major taxonomic groups, except for "terrestrial animals" which included flying adults of aquatic organisms and any other organisms assumed to have been captured on the surface. Where food could not be counted (hatchery food, plant material), it was given a count of 1.

Product moment correlation coefficients (r) were used as the measure of food similarity, and were subsequently transformed to standard normal deviates (Z) for comparisons. Where r was 1.0, it was given the value of 0.99 to avoid zero division. Where r was undefined because one stomach content in a comparison had no food, or had equal quantities of all foods, the data were omitted from analysis.

Correlation coefficients for stomach contents from the same individual ("within" comparisons) were calculated from consecutive pairs of stomach content samples. Where the first and second samples are taken as the first pair, the second and third as a second pair, and the first and third samples as a third pair, the comparisons are nonorthogonal. In the main analysis (Table 2) no more than two comparisons involved the same samples, thus making the comparisons approximately orthogonal. Correlation coefficients were only calculated when two samples were taken within a specified time interval, which varied for different analyses, and were not calculated for samples from fish that had moved in the time interval from Section 1 to Section 2 or 3 (Fig. 1), or vice versa.

Correlation coefficients for stomach contents from different individuals ("between comparisons") were calculated from pairs of samples, one of which was from the "within comparisons" and the "other" from a trout of the same species, similar length (within 15%), from the same section (1, 2, or 3; Fig. 1), captured at the same time. Because the "other" might be any one of several, all possible comparisons could be made but, again, this would make the comparisons nonorthogonal and, in the main analysis, comparisons were so restricted as to make them approximately orthogonal.

After transformation of the correlation coefficients to standard normal deviates, differences between the mean Z values for the two groups "within" and "between" were evaluated using an unpaired t test. All standard errors were estimated on calculated rather than theoretical variances.

Food of Individuals in Repeated Sampling

For all three species of trout captured in the stream and rainbow trout in the pond, food samples from the same individuals ("within comparisons") were more similar than food samples from different

TABLE 2. Mean values of food similarity (r converted to Z) for samples from the same individual ("within" comparisons) and samples from different individuals ("between" comparisons). Maximum interval between samples was 21 days; mean, 11.4 days.

Species	Within comparisons: Mean Z_W	n	Between comparisons: Mean Z_B	n	$Z_W - Z_B$	Probability
Rainbow trout (pond)	–0.15	11	–0.47	48	0.31	.20–.30
Rainbow trout	0.82	56	0.45	247	0.37	.025–.05
Cutthroat trout	0.71	20	–0.13	50	0.84	.01–.025
Brook trout	1.33	5	0.23	11	1.10	.10–.20
Total	0.71	92	0.08	356	0.63	.001

individuals (Table 2). The same outcome was obtained when similarities were measured using weights of the food items. The difference between the Z value for "within" (Z_W) and that for "between" (Z_B) is a measure of food specialization. In the aggregate of the data of Table 2, the degree of specialization is 0.63 on a theoretical scale, which 99% of the time would extend from −6 to +6.

Individuals that showed high or low Z_W values were not typically those that had eaten unusual kinds of foods. An exception was a trout from the pond which was the only one to have eaten salamanders, which had done so on three occasions, and which did so when given a choice of foods in a laboratory situation. In general, the full variety of kinds of foods was eaten both by individuals that showed positive specialization ($Z_W > Z_B$) and by individuals that showed negative specialization ($Z_W < Z_B$) (Fig. 2). This implies that different individuals specialized on selections from many of the foods. Minor differences among the distributions may nevertheless have biological significance. Types of food that were probably drifting in the stream (diptera pupae, fish eggs, ephemeroptera naiads, terrestrial animals) were usually eaten to a greater extent by trout with negative specialization measures. Kinds of food presumably obtained by foraging (gastropoda, fish) were eaten more frequently by trout with positive specialization. These generalizations apply equally well to the individual species. A predominance of chironomid larvae, a forage food, was shown in cutthroat and brook trout with positive specialization measures, but not in rainbow trout nor in the aggregate shown in Fig. 2.

Specialization, either positive or negative, was not associated with a discrete group of individual trout. Values of Z_W and values of ($Z_W - Z_B$) had unimodal distributions with a mean slightly greater

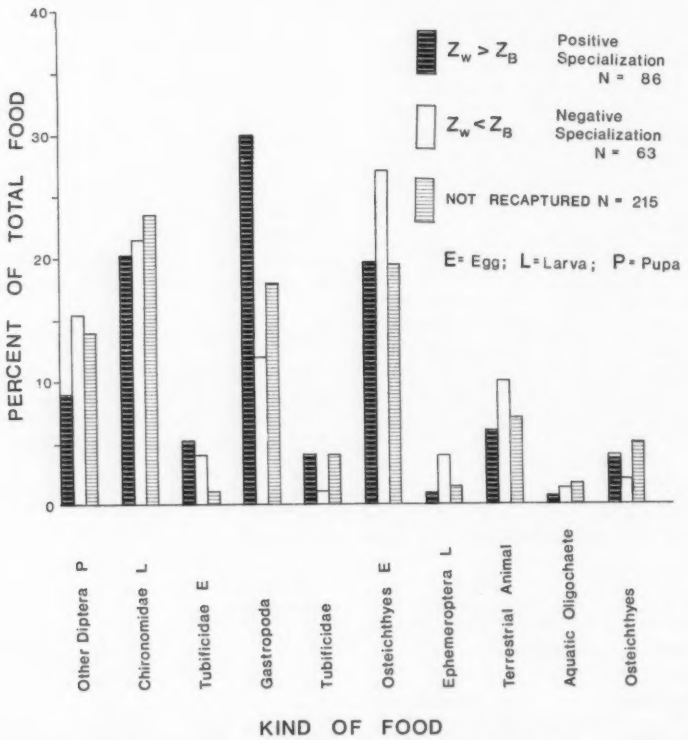

FIG. 2. Food eaten by trout with positive or negative values of $Z_W - Z_B$ compared with food of those which were not recaptured within 21 days. The data are for all three species.

than zero. Moreover, individuals captured several times did not have values that were characteristically high or low.

Degree of specialization was highest over short time intervals between capture and recapture (Fig. 3), declined to zero for intervals between 20 and 50 days, and varied upward beyond that, suggesting the possibility of residual specialization at intervals of 60–180 days. Statistical tests that might be suggested by the standard errors of the estimates are of dubious value because the comparisons are not orthogonal (to make them orthogonal would have sacrificed too much information). Nevertheless, a long duration of specialization is a possibility. Feeding opportunities seemed stable and many kinds of food were eaten throughout the year (Fig. 4). There was no seasonal trend in the value of $(Z_W - Z_B)$.

Values of Z_W and of $(Z_W - Z_B)$ showed poor correlation with total weight of food, numbers of kinds of food items, growth rate, or length of the trout (Table 3). The slight positive correlations between recapture interval and Z value, and recapture interval and value of $(Z_W - Z_B)$, reflects that the data were blocked by time to make comparisons homogeneous. Although some of the correlations with other variates were significant, none accounted for more than 10% of the variation ($r^2 < 0.1$). The most noteworthy correlations were those with the number of kinds of food items. The more kinds of food eaten, the higher the values of Z_W and of $(Z_W - Z_B)$. Weight and number of kinds of food were correlated with growth rate.

Fig. 5 indicates that trout caught in different subsections had eaten kinds of food items in different proportions. Consequently, the specialization analyses were repeated on trout recaptured in the same and different subsections. Surprisingly, the degree of food specialization was less for trout recaptured in the same subsection (0.64) than for those recaptured in different subsections (0.85), but the difference was not statistically significant. This indicates that it was not necessary for trout to feed in the same area to obtain food similar to that eaten previously.

Experimental Observation of Foods Eaten by Trout

Materials and Methods

An experiment was designed to test the same hypotheses as in the foregoing: are the foods eaten by an individual trout at different times more similar than the foods eaten by different trout at different times? Four small ponds, each 61 cm high, 84 cm wide, and 175 cm long, were filled to a depth of 51 cm. To each was added a substratum and a density of food organisms that approximated the natural condition in Fishing Pond (Fig. 1). Each pond was divided into three equal-sized sections connected by passageways, an arrangement that helped give the ponds more spatial complexity. Four individually fin-clipped rainbow trout, approximately 50 mm fork length, were placed in each pond as permanent residents, and these were called the "resident" trout because they were continuously in an environment to which they could presumably become accustomed. They had previously only experienced hatchery food in holding aquaria. Stomach content samples were taken from the "resident" trout at various intervals. Each sample was obtained by stomach flushing with a hypodermic syringe and was preserved in 10% formalin. An hour after each time of sampling, the "resident" trout were removed and were replaced by four "naive" trout from the same holding aquaria where the "resident" trout had originated. Feeding by the "naive" trout was permitted for 2 hr; they were then removed and the "resident" trout were returned 1 hr later. Each "naive" trout was used only once. For estimating weights of food eaten, foods in samples were combined by periods of 8 days, dried

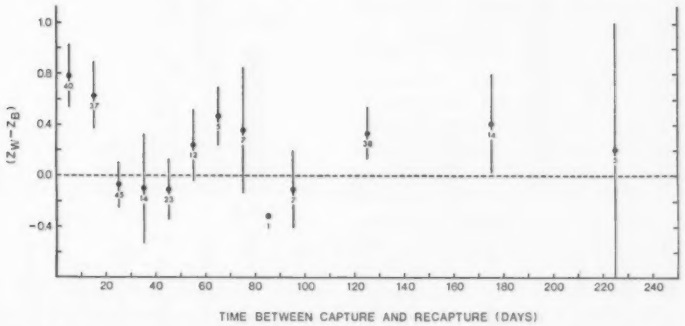

FIG. 3. Value of $Z_W - Z_B$ for three trout species over different intervals of capture. Standard errors are indicated by vertical bars, and the number of "within" comparisons is written below each point.

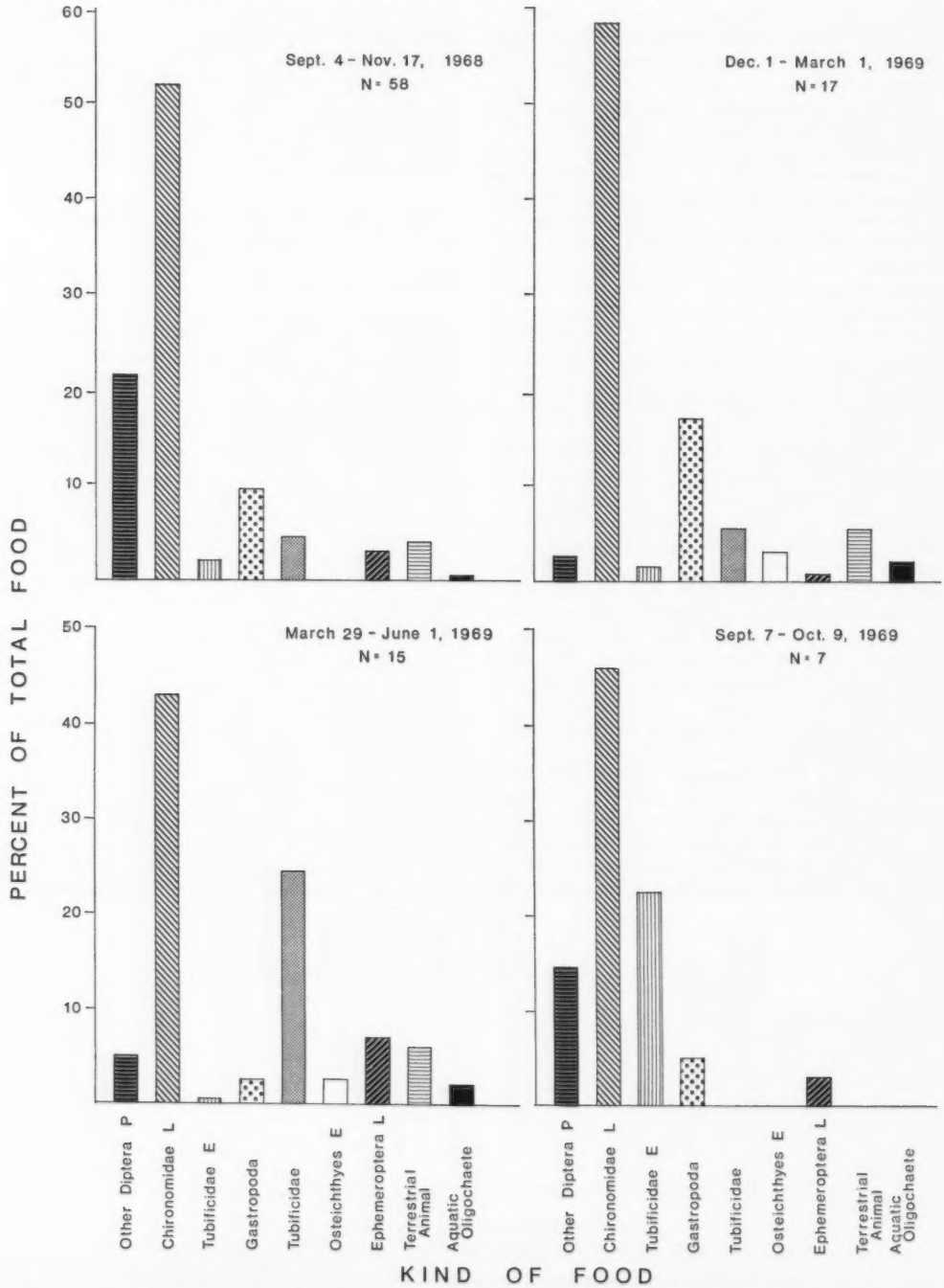

FIG. 4. Food eaten by all cutthroat and rainbow trout captured in subsection 2B of Marshall Creek at different seasons during 1968 and 1969.

TABLE 3. Correlations between Z_W values, (Z_W–Z_B) values, and various characteristics of recaptured trout. All comparisons were blocked by species and by the time interval between captures (1–20, 21–50 and 51–200 days). Data on weight of food were for rainbow trout only (176 observations); otherwise data were for all trout (239 observations). Variates: R = recapture interval, N_1 = number of food items at capture, N_2 = number of food items at recapture, NT = total number of food items in two captures, W_1/L = weight of food at capture divided by log length, W_2/L = weight of food at recapture divided by log length, W_T/L = weight of food at capture plus recapture, all divided by log of average length, G = mean daily growth in length, and L = log length at recapture. The symbol A signified that the variates were not mathematically independent and the correlation therefore not calculated.

	Variates										
	Z_W	Z_W–Z_B	R	N_1	N_2	NT	W_1/L	W_2/L	WT/L	G	L
Z_W	–										
Z_W–Z_B	A	–									
R	.028	.018	–								
N_1	.182[a]	.078	–.154	–							
N_2	.164[a]	.101	–.002	.175[a]	–						
NT	.226[a]	.117	–.102	A	A	–					
W_1/L	.176[a]	.143[a]	–.102	.328[a]	.080	.273[a]	–				
W_2/L	–.039	–.033	–.016	.241[a]	.342[a]	.385[a]	.205[a]	–			
WT/L	.084	.067	–.075	.365[a]	.277[a]	.426[a]	A	A	–		
G	.055	.050	–.028	.187[a]	.170[a]	.233[a]	.088	.195[a]	.184[a]	–	
L	.183[a]	.173[a]	–.040	.223[a]	.162[a]	.251[a]	.242[a]	.249[a]	.317[a]	.010	–

[a]Significance at $P < .05$.

(16 hr at 50 C), and used to obtain conversion factors for numbers to weight. Some direct observations were made of feeding behavior in the ponds. For this purpose, "resident" trout were removed from a pond and held overnight without food, returned to the pond in the morning, and observed for four 3-min periods. The entire experiment was replicated twice, once in June and once in August; the details of each replication were slightly different (The domestic rainbow stock was from Trout Lodge Springs Inc., Tacoma, Washington; the wild stock was from Loon Lake, B.C.):

	June 2–26 Domestic	Aug. 8–Sept. 3 Wild
Age (*months*)	12	22
Initial fork length (*mm*)	63–78	43–48
No. observation times	8	10
No. ponds	3	4
No. kinds of food analyzed	8	5
Days food sampled (from start)	1,3,5,7,9, 11,17,24	1,3,5,7,9,11, 13,17,19,26
Days behavior observed	none	9,11,13

RESULTS

The focus in the experiment being on food specialization, the main questions to ask in the analysis are whether the quantity and variety of food eaten changes with time in a different way in "naive" and "resident" fish. For "naive" trout there was no significant change in weight of food in the stomach with time (i.e., the time effect was not significant), implying that there was the same amount of food present throughout the experiment (Table 4). Importantly, for "naive" fish, the interaction (KT) between kind of food (K) and time (T) was not significant in either replication. This implies that the relative abundance of different kinds of prey was constant.

For "resident" trout there was a significant interaction between kind of food and time in both replications (Table 4). This can only be interpreted to mean that there was some degree of specialization by the group of "resident" fish as a whole. The significant interaction between kind of food and individuals (IK) indicates that different individuals selected different kinds of prey, thus showing specialization. The significance of other interactions suggests that the ponds (P) were utilized for food in various ways by the trout as groups and individuals during the experiment. This is not surprising considering the difficulties of making quasi-natural environments similar and of obtaining comparable individuals and groups of trout. These results are not accounted for by highly unusual choice of particular kinds of food by particular individuals at particular times. Most individuals showed only a slight consistency in their choice of food items over a series of days. In consequence, the experimental results reflect small but significant amounts of specialization by most individual trout.

This is illustrated by Fig. 6 which presents examples of food eaten by individuals in two ponds. The data are based on numbers instead of weights and the category "other" was omitted from analyses of variance, as was "diptera pupa" in the June replication. The varieties and degrees of specialization shown by the various individual trout are not easily summarized. In some ponds, most individuals ate characteristic assortments of food. In other ponds, there was less individual consistency and less difference among individuals. Most individuals had some items which consistently predominated in the food, but other items were added in varying amounts at the different times. The data are suggestive of various degrees and duration of specialization, presumably reflecting individual differences and current success of various feeding techniques.

TABLE 4. Statistically significant effects ($P < .05$) in the small pond experiment. K = kind of food, P = pond, T = time.

		Naive	Resident
June Replication	Main effects	None	K, P
	Interaction	None	IK, IP, KP, KT, IKP, KPT
August Replication	Main effects	K, P	K, T
	Interaction	KP	IK, KP, KT, IKT, KPT

Accelerated swims toward the object culminating with ingestion ("strikes") were the most conspicuous aspect of feeding behavior, and there was no single well-defined searching pattern. Although agonistic behavior, as described by Stringer and Hoar (1955), occurred frequently, the trout moved freely about the pond with no apparent site attachment, that is, they were aggressive but not territorial. Individuals did not strike with the same frequency at objects in different strata in the ponds (substratum, sur-

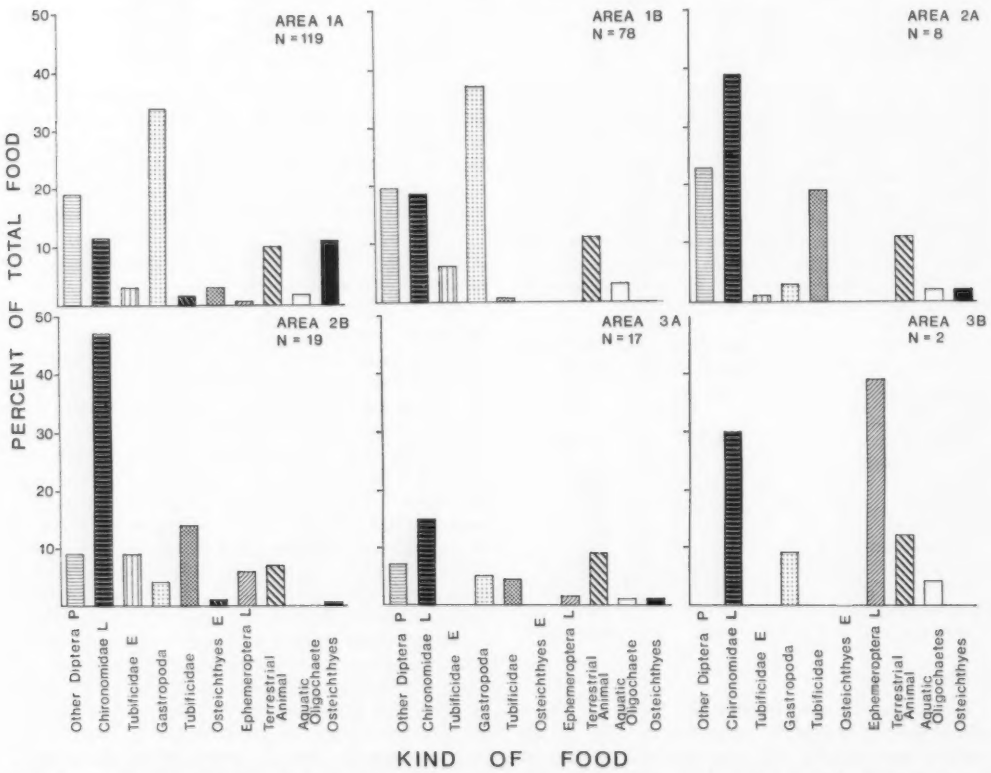

FIG. 5. Food eaten by all cutthroat and rainbow trout captured in different subsections of Marshall Creek between April 10 and October 9, 1969.

face, water column, wall; $\chi^2 = 74.2$; df = 21; $P < .001$).

Discussion

The foregoing provides evidence that, in field situations, individual trout demonstrate differences in the food they eat and that these differences persist to result in specialization. Thus, individual trout eat only part of the entire spectrum of prey types consumed by all individuals of the population.

The degree of food specialization by trout in natural situations is difficult to judge because the schemes of food classification which investigators use may differ from those which trout use. The categories of food items which we used were based on convenience and what we would think were meaningful categories to trout. From a trout's point of view, the categories were probably in some degree lumped when they should have been split, and vice versa. Moreover, because it was necessary to handle the fish and to pump out their stomach contents, the interval between sampling periods was relatively long. Our results may thus be indicative of much more specialized feeding than the analyses suggest.

Prey specialization may occur in a variety of predators. Tinbergen (1960) presented data on food captured by great and blue tits in a pine wood which suggested that individuals specialized on particular prey species. Unfortunately, the data were presented for less than 10% of the birds studied; consequently, it is not possible to determine whether

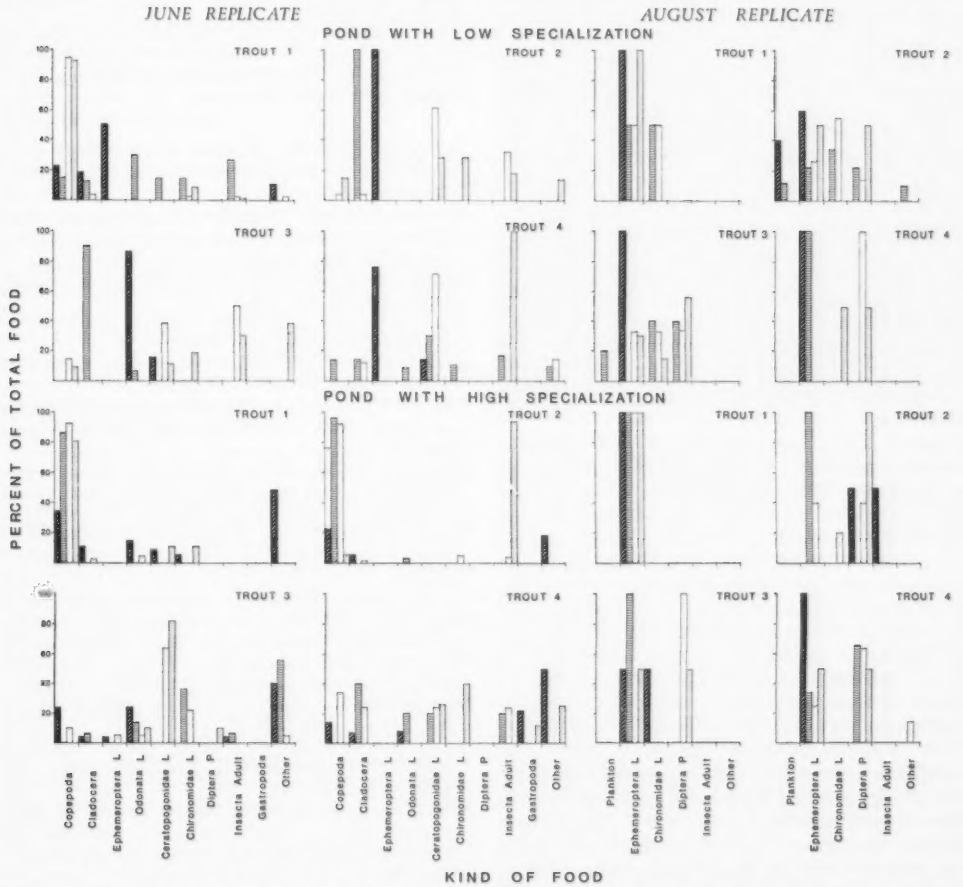

FIG. 6. Food eaten by individual rainbow trout in ponds where low or high specialization was apparent during the first week. Day 1, heavy diagonal shading; Day 3, horizontal lines; Day 5, no shading; Day 7, grey.

specialization is typical of these predators. Heterogeneity in food eaten by animals captured at nearly the same time and place has been intimated for salmon, carp, sunfish, salamanders, and gulls (Allen 1941; Ivlev 1961; Reed MS 1971; Neish MS 1970; and Harris 1965). Such heterogeneity may occur without food specialiazation. It may result because prey distributions were contagious in space or time, or because of rapid conditioning as observed in crows (Croze 1970). It is also possible that food heterogeneity and food specialization are usually related phenomena as in trout.

Food specialization has also been observed among individual animals offered excess food in the laboratory. Brown (1969) found that pigeons ate different kinds of seeds in proportions characteristic for an individual. Such food specialization may not result from the same cause(s) as specialization in the field. As prey location and capture were simplified in the laboratory, the individual differences might merely reflect differences in food "preference."

In field situations, on the other hand, the cause of food specialization is likely to be more complicated. In "natural" populations of trout, food specialization probably results from individual differences in the probability of detecting a particular kind of prey. These individual differences seem to result from differences in conditioned learning of two sorts: strikes in response to different stimuli and development of different modes of feeding behavior. In the small pond experiment, individuals fed in different pond strata with unequal frequency, presumably reflecting individual differences in stimuli used for prey detection. Field observations indicated that individuals had slightly different searching behavior; indeed, individual differences in searching technique, capturing technique, and feeding rhythm probably contribute to food specialization in trout.

Food specialization may have important consequences for both predator and prey. Presumably, specialization is adaptive and may comprise for the predator a useful stratagem for exploiting to advantage the spatial and temporal variations in natural environments. When and where a particular species of prey is abundant, a predator may find it advantageous to temporarily perfect an appropriate catching technique. Murdoch (1969) has considered some of the possible consequences for prey of this kind of predator food specialization. When a prey species is particularly abundant, a predator may attack it disproportionately. When the prey becomes scarce, the predator may "switch" the great proportion of its attacks "to another prey which has become the most abundant." In consequence, the predator may have a stabilizing effect on an animal community structure. Whether this is true in Marshall Creek, or in other natural environments which trout inhabit, is open to conjecture. However, it seems likely that, as one of many potential predators, trout may contribute to community stability.

Acknowledgments

We are especially grateful to N. E. Gilbert for suggestions about statistical analysis. The following persons assisted with the field work at different times: R. Babb, W. R. Black, M. B. Lambert, D. L. Kramer, A. I. D. Maurer, J. S. Stimson, A. F. Tautz, and B. Wong. G. S. Jamieson, G. G. E. Scudder, and K. Stewart helped identify insect larvae. C. S. Holling, M. H. Keenleyside, J. D. McPhail, and T. G. Northcote criticized the manuscript. S. W. Borden and D. Laurient gave advice on programming.

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ALLEN, K. R. 1941. Studies on the biology of the early stages of the salmon (*Salmo salar*). J. Anim. Ecol. 10: 47–76.

BELL, G. R. 1964. A guide to the properties, characteristics, and uses of some general anaesthetics for fish. Fish. Res. Board Can. Bull. 148: 4 p.

BROWN, R. G. B. 1969. Seed selection by pigeons. Behaviour 34: 115–131.

CROZE, H. 1970. Searching image in carrion crows. Z. Tierpsychol., Suppl. 5: 86 p.

HARRIS, M. P. 1965. The food of some *Larus* gulls. Ibis 107: 34–53.

IVLEV, V. S. 1961. Experimental ecology of the feeding of fishes. Yale Univ. Press, New Haven, Conn. p. 66–72.

MURDOCH, W. W. 1969. Switching in general predators: experiments on predator specificity and stability of prey populations. Ecol. Monogr. 39: 335–354.

NEISH, I. C. MS 1970. A comparative analysis of the feeding behaviour of two salamander populations in Marion Lake, B.C. Ph.D. Thesis, Dep. Zool., Univ. British Columbia, Vancouver, B.C. 108 p.

REED, R. C. MS 1971. An experimental study of prey selection and regulatory capacity of bluegill sunfish (*Lepomis macrochirus*). M.A. Thesis, Dep. Biol., Univ. California, Santa Barbara, Calif. 106 p.

SEABURG, K. G. 1957. A stomach sampler for live fish. Progr. Fish-Cult. 19: 137–139.

STRINGER, G. E., AND W. S. HOAR. 1955. Aggressive behavior of underyearling Kamloops trout. Can. J. Zool. 33: 148–160.

TINBERGEN, L. 1960. The natural control of insects in pinewoods. I. Factors influencing the intensity of predation by songbirds. Arch. Neer. Zool. 13: 265–343.

WINDELL, J. T., AND D. P. NORRIS. 1969. Gastric digestion and evacuation in rainbow trout. Progr. Fish-Cult. 31: 20–26.

OBITUARY

Andrew William Lantz

1905-1972

A. William Lantz's many friends and colleagues were shocked and deeply grieved to learn of his death in British Columbia on February 10, 1972. Not only did countless Canadians in all walks of life know and respect "Bill" Lantz, but he acquired an enviable international reputation as a friend and as a scientist.

He was born in Calgary, Alberta, on October 5, 1905, and received his academic training from the University of Alberta. His long and distinguished career in food science began with 7 years association with the meat packing industry where he retained a lasting affiliation and interest. He then directed his attention to fisheries wherein he made his most noteworthy contributions.

Bill began his 23 years of meritorious service with the Fisheries Research Board by joining the Vancouver Laboratory in 1944. His work there on refrigerated storage and transport of fishery products and the mechanical handling and smoking of fish had a very significant impact on Canadian fisheries. These and other contributions were soon recognized internationally and during 1953–56 he was granted leave of absence to become Director of a Colombo Plan project in Ceylon, wherein he successfully introduced new fishing and processing methods and directed the construction of a harbor and a modern fish storage and processing plant.

On returning to the Fisheries Research Board in 1957, Bill moved to London, Ontario, where he initiated pioneering work related to our freshwater fisheries. His accomplishments in this area are far too numerous to mention but he can justifiably be considered the "father of freshwater fisheries technology" in Canada. We have yet to reap the full benefit of his endeavors inasmuch as some are only now being exploited. Nevertheless, his impact was very real in that he encouraged and demonstrated new uses for underexploited species, the need for modern facilities and methods, and the introduction of new products like canned whitefish and smoked Arctic char. He had a large number of firsts to his credit and his book, "Special Products from Freshwater Fish," was an outs anding success both here and abroad.

When the Fisheries Research Board moved its operations from London to Winnipeg in 1966, Bill continued to direct and participate in investigations associated with freshwater fish products and processing in the newly established Freshwater Institute. He also assumed the responsibility of Industrial Liaison Officer for the Institute, a position that brought him in contact with all segments of Canada's freshwater fisheries.

Although Bill Lantz retired from the Fisheries Research Board in July of 1970 and he and Mrs Lantz moved to Chilliwack, B.C., he was still very active and in demand as a consultant. Just prior to his retirement he became the third recipient of the W. J. Eva Award of the Canadian Institute of Food Science and Technology for his outstanding contributions to food science and technology.

It is difficult to say so little about a man that did so much. Bill Lantz was truly a distinguished Canadian scientist who had a special talent for communicating with people and who always had the practical approach in mind. He shall certainly not be forgotten.

E. G. Bligh

NOTES

Acidification and Bubbling as an Alternative to Filtration in Determining Phytoplankton Production by the ^{14}C Method

D. W. SCHINDLER, R. V. SCHMIDT, AND R. A. REID

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SCHINDLER, D. W., R. V. SCHMIDT, AND R. REID. 1972. Acidification and bubbling as an alternative to filtration in determining phytoplankton production by the ^{14}C method. J. Fish. Res. Bd. Canada 29: 1627–1631.

Acidification and bubbling was found superior to membrane-filtration for post-incubation treatment of ^{14}C-productivity samples. The method eliminates errors associated with filtration. Results are compared with those of conventional and corrected filtration procedures, and application of the method is discussed.

SCHINDLER, D. W., R. V. SCHMIDT, AND R. REID. 1972. Acidification and bubbling as an alternative to filtration in determining phytoplankton production by the ^{14}C method. J. Fish. Res. Bd. Canada 29: 1627–1631.

Dans des études de productivité par la méthode du ^{14}C, nous avons utilisé l'acidification et le barbottage au lieu du filtrage sur membrane pour le traitement post-incubatoire des échantillons. Cette méthode élimine les erreurs inhérentes au filtrage. Nous comparons les résultats de cette technique avec ceux du filtrage conventionnel ou corrigé, et discutons de son application.

Received February 28, 1972

MEMBRANE filtration has proved to be a complicating factor in the measurement of phytoplankton production by the ^{14}C method. Arthur and Rigler (1967) first pointed out that the activity retained on the filter per unit volume filtered increased as smaller and smaller volumes were filtered. Also, they hypothesized that, with increasing volumes filtered, cell breakage on filtration increased. Since dissolved organic matter found in filtrate has commonly been referred to as "extracellular products," which are normally thought to be excretory products of phytoplankton, Arthur and Rigler's finding cast considerable doubt on the method for partitioning phytoplankton production into "particulate" and "dissolved" components.

More recently, Schindler (1972) found that the sum of activity in filtrate and on filters agreed well with activities of unfiltered lake water in incubation bottles, and that the proportion of activity retained on the filter was reciprocally related to volume filtered. The concentration of labelled organic material in filtrate, however, remained constant regardless of volume filtered. Still more recently Nalewajko and Lean (1972) found that in some instances, retention of dissolved substances containing both inorganic and organic forms of ^{14}C by membrane filters could produce a false correction for filtration error. The labelled material retained by filters was in excess of that expected due to activity of water alone. The effect was particularly pronounced when low volumes were filtered. Their results, while casting some doubt on the Arthur–Rigler (1967) correction procedure, also failed to entirely explain the observed correction for filtration error. The absence of conclusive proof has led us (Schindler 1972; Schindler and Nighswander 1970) to publish both corrected and uncorrected values for our recent primary production analyses.

Because the amount of "excess" radioactive material retained by filters appears to vary from lake to lake and is *p*H dependent (Nalewajko and Lean 1972), membrane filtration does not appear to give primary production results which may be validly compared from one lake to another. The major purpose of this paper is to outline a method which, in combination with modern liquid scintillation counting procedures, may serve as a convenient alternative to filtration in ^{14}C production measurements. A test for validity is also given. Fogg (in Vollenweider 1969) described the use of acid-

Printed in Canada (J2458)

ification and bubbling for determining extracellular-product release by phytoplankton using the ^{14}C method. This paper describes apparatus and techniques which may be used to extend the technique, so that it may be conveniently used to measure uptake of total carbon by phytoplankton in photosynthesis.

The technique — The apparatus described below (Fig. 1) can be used to process 21 samples simultaneously. It can easily be adapted for larger or smaller numbers. The device consists of twenty-one short 30 ml Allihn filter tubes with fine fritted glass discs at the base (Kimble Products), connected to the pressure side of a Millipore pump using lengths of Tygon tubing and a manifold of aquarium valves. The tubes were mounted in seven rows of three tubes each, using a plexiglass frame.

To use the apparatus, phytoplankton production incubations are performed as described by Schindler and Holmgren (1971). After incubation 0.2 ml of 0.1N HCl is injected into each column,[1] the filter pump is started, and approximately 20 ml from each incubation bottle is placed in a column and bubbled rapidly with air for 20 min. After bubbling, 2.5 ml are taken from each filter tube and placed in a liquid scintillation vial for counting. Fifteen milliliters of dioxane-base fluor (Schindler and Holmgren 1971) are added to each vial for counting. All samples were counted using a Picker Liquimat 220 scintillation counter and the external-standard channels ratio method for calibration. Counting efficiency is approximately 70%.

Phytoplankton production (C_0 in mg C/liter) is calculated from the acidification and bubbling results as follows:

$$C_o = \frac{(C_1)(^{14}C_a)\left(\frac{V_b}{V_a}\right)}{^{14}C_b}$$

where C_1 is the concentration of dissolved inorganic carbon (mg/liter), $^{14}C_a$ is the activity of ^{14}C in the aliquot counted (dpm), $^{14}C_b$ is the activity of ^{14}C added to the incubation bottle (dpm), V_b is the volume of the incubation bottle (ml), and V_a is the volume of the aliquot counted (ml).

Tests for efficiency — The technique was applied to distilled water to which a known amount of $^{14}CO_3^=$ had been added. The procedure drove off 99.995% of the inorganic ^{14}C. We believed, however, that there was some danger of labelled HCO_3^- or $CO_3^=$ binding directly to humic material or "colloids" in lake water, which would tend to make the method overestimate primary production. We tested this possibility as follows:

A standard primary production sample was acidified and bubbled as described above after a 4-hr in situ incubation. Immediately after acidification and bubbling, a 2.5 ml aliquot was titrated back to lake *p*H with NaOH,

[1]Acid of greater strength may be required for waters of higher buffering capacity. The *p*H during bubbling should be 3–3.5 to ensure complete stripping of dissolved inorganic carbon.

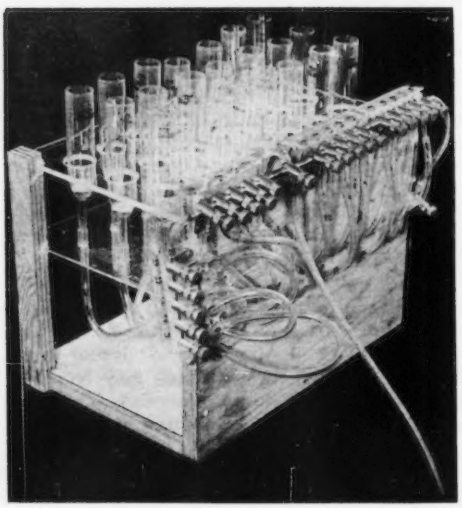

FIG. 1. The apparatus for acidification and bubbling of incubated samples.

TABLE 1. Distribution of ^{14}C activity in a post-incubation sample from a depth of ½ m in a Canadian Shield lake (Lake 304), May 17, 1972. The sample was acidified and bubbled as described in the text, then filtered through a Millipore HA membrane and injected on G25 Sephadex.

	% of total activity	
	Before acidification and bubbling	After acidification and bubbling
Filterable[a]	24.0	95.0
High molecular weight (>5000)[b]	0.8	3.5
Low molecular weight (<100)[b]	0.3	1.5
Dissolved inorganic carbon[c]	74.9	–

[a]Activity removed by 25 mm Millipore HA membrane.
[b]Sephadex fractions give apparent molecular weight. The low molecular weight fraction probably includes HCO_3^-, $CO_3^=$, and glycollate or any other low molecular weight organic compounds.
[c]Fraction removed by acidification and bubbling.

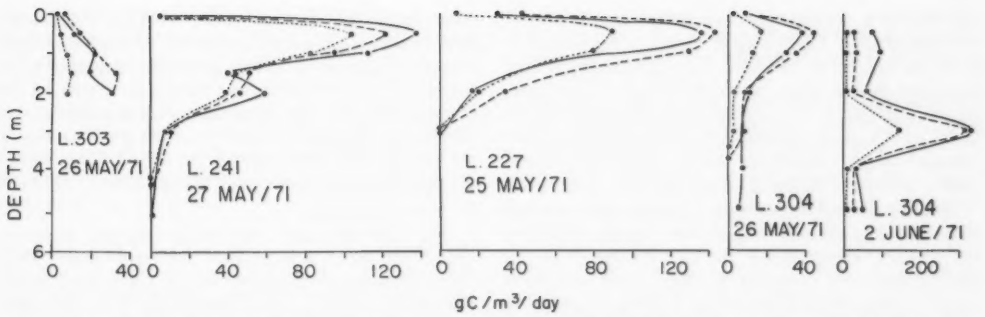

FIG. 2. Phytoplankton production measurements obtained for several Canadian Shield lakes by acidification and bubbling (———), filtering without correction for filtration error (- - - - - - -), and filtering with correction (Arthur and Rigler 1967) for filtration error (— — — — — —).

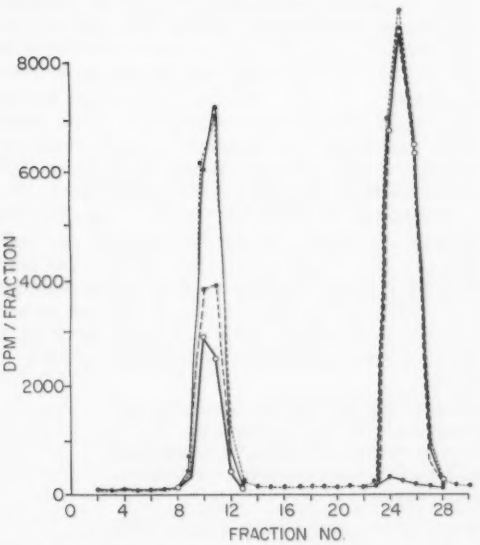

FIG. 3. Analysis of ^{14}C-equilibrated lake water by Sephadex G25 after filtering different aliquot sizes through Millipore HA (0.45 .) membrane. ●- - -● 5 ml; ●——● 5 ml, acidified and bubbled before filtration; ●— —● 2 ml; ○———○ 1 ml. The peak on the left (void volume or molecular weight 5000 by Sephadex-proved to be molecular weight 10^6 to 10^7 i.e. colloidal in subsequent analysis. Note that the amount of this peak in filtrate is proportional to the volume filtered. The peak on the right (apparent molecular weight <100) consists of dissolved inorganic carbon and small organic ecretory products. Note that 5 ml after acidification and bubbling had very little material present, i.e. small molecular excretory products such as glycollate were not abundantl An analysis performed on filtrate from a 25 ml fraction was identical to that of the 5 ml fraction shown. See Table 2 for radioisotope balance.

filtered (Millipore HA, 25 mm diameter, low vacuum)[2] and quickly injected onto a column of G25 Sephadex gel. This method has proven useful in separation of radioactive-labelled dissolved compounds in lake water (Lean 1972; D. R. S. Lean and D. W. Schindler unpublished data). The products of gel-filtration were collected in 40 fractions on a fraction collector and radioassayed with a liquid scintillation counter and the above-mentioned fluor.

Comparison with filtration methods — In several experiments from different lakes, 50-ml subsamples were filtered onto Millipore HA membranes 47 mm in diameter. Corrections for filtration error (Arthur and Rigler 1967) were also performed by filtering 25, 10, 5, 2, and 1 ml aliquots. An additional aliquot was acidified and bubbled as described above.

Results — Only a tiny fraction of labelled HCO_3^--size material remained in lake water after acidification and bubbling (Table 1), so that this appears to be an insignificant source of error in our method.

Total carbon uptake by phytoplankton as measured by the acidification and bubbling method was consistently higher than that measured by the filtration method, uncorrected for filtration error (Fig. 2). When corrected for filtration error, filtered results were similar to bubbled ones.

This rather good agreement between the bubbling method and corrected filtration results was puzzling at first, since Nalewajko and Lean (1972) had suggested that the correction for filtration error was an artifact due to retention of colloidal and dissolved labelled material by membrane filters. We therefore

[2]While filtration is undesirable in this test if the findings of Nalewajko and Lean (1972) are correct, it is necessary before samples are partitioned by Sephadex. Based on total activity on the filter, the possible error introduced was extremely small in this experiment.

performed a second Sephadex experiment to test this possibility. In this experiment, aliquots of 1, 2, 5, and 25 ml were taken from a sample of lake water which had been incubated for 24 hr with dissolved inorganic ^{14}C. These were filtered through 0.45 μ Millipore membranes and the filtrate was injected onto Sephadex G25. Radioactivity of filters and all Sephadex fractions was assayed.

Results of this experiment indicate that colloid retention was entirely responsible for the filtration correction. When only 1 ml was filtered, most of the colloid was removed by the membrane filter (Fig. 3). As larger volumes were filtered, progressively more colloidal material passed through the membranes. Dissolved inorganic carbon retention was equal in all cases.

A complete radioactivity balance is given in Table 2. When filter activities in dpm/ml (column 3) were plotted against volume filtered, a typical filtration correction curve resulted. The increase in radioactivity/ml with decreased volume filtered accounted for almost all of the colloid radioactivity missing from colloid in Sephadex analyses.

Discussion — The colloidal fraction does not become radioactive when incubated with dissolved inorganic ^{14}C in the absence of plankton, indicating that the process is biologically mediated (Lean 1972, D. R. S. Lean and D. W. Schindler unpublished data). It therefore appears that colloid radioactivity should be included in production estimates. This can be done with minimum effort by our acidification and bubbling technique, but also by carefully-done corrections for filtration error. Since Nalewajko and Lean (1972) have found that filters may retain dissolved inorganic carbon as well as organic forms, filters should be thoroughly fumed as a part of the filtration correction procedure if the retention of dissolved inorganic carbon by filters is great enough to warrant this procedure. Our tests indicate that all dissolved inorganic ^{14}C is removed from filters by this procedure.

The labelled low molecular weight material remaining after acidification and bubbling (Table 1) is not necessarily bicarbonate, since other small molecular weight compounds (for example, glycollate, see Fogg et al. 1965) would not be distinguished by the technique used. The amount of radioactivity retained is, however, too low for this to be of major concern here.

The amounts of extracellular products reported in past studies must be viewed with distrust. The normal procedure for analyzing extracellular products formed by primary production is to filter incubated lake water and then acidify and bubble the filtrate. Since the membrane filter would retain both suspended and dissolved organic forms of labelled carbon, the percentage extracellular products found by past investigators may be too low.

The source of extracellular products, i.e. dissolved compounds which cannot be removed by acidification and bubbling, remains obscure. The high molecular weight material could be due to breakage on filtration or to direct binding of excreted carbon by colloidal materials, but it is probably too large to be an excretory product. Until further evidence is

TABLE 2. Distribution of radioactivity in samples of different volumes filtered through Millipore HA (0.45 μ) membrane, then run through Sephadex G-25. A Sephadex analysis of an acidified and bubbled sample is shown for comparison. Colloid (M.W.> 5000) and inorganic (M.W.< 100) activities are calculated by integrating appropriate areas of Fig. 3. All activities are rounded to the nearest hundred dpm.

Treatment	Filtrate		Filter (*dpm/ml*)	Total	recovery
	Colloid	Inorganic			
None (5 ml unfiltered)	–	–	–	51700	100
5 ml filtered through 25 mm diameter membrane	16400	24100	9900	50400	98
2 ml filtered through 25 mm diameter membrane	10100	24100	15400	49600	96
1 ml filtered through 25 mm diameter membrane	6900	24100	20800	51800	100
25 ml filtered through 47 mm diameter membrane	14700	24100	9000	47800	93
5 ml filtered, then acidified and bubbled	16755	100	10100	26900	52
5 ml acidified and bubbled	–	–	–	26400	–

supplied, we believe that total production by our method offers the safest means of assessing total carbon uptake by phytoplankton.

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ARTHUR, C. R., AND F. H. RIGLER. 1967. A possible source of error in the ^{14}C method of measuring primary productivity. Limnol. Oceanogr. 12: 121–124.

FOGG, G. E., C. NALEWAJKO, AND W. D. WATT. 1965. Extracellular products of phytoplankton photosynthesis. Proc. Roy. Soc. London B Biol. Sci. 162: 517–534.

LEAN, D. R. S. 1972. Phosphorus compartments in lake water. Ph.D. Thesis. Univ. of Toronto, Toronto, Ont.

NALEWAJKO, C., AND D. LEAN. 1972. Retention of dissolved compounds by membrane filters as an error in the ^{14}C method of primary production measurement. J. Phycol. 7: 37–43.

SCHINDLER, D. W. 1972. Production of phytoplankton and zooplankton in Canadian Shield lakes. Proceedings, IBP-UNESCO symposium on productivity problems of freshwaters. Pol. Acad. Sci. (In press)

SCHINDLER, D. W., AND S. K. HOLMGREN. 1971. Primary production and phytoplankton in the Experimental Lakes Area, Northwestern Ontario, and other low-carbonate waters, and a liquid scintillation method for determining ^{14}C activity in photosynthesis. J. Fish. Res. Bd. Canada 28: 189–201.

SCHINDLER, D. W., AND J. E. NIGHSWANDER. 1970. Nutrient supply and primary production in Clear Lake, Eastern Ontario. J. Fish. Res. Bd. Canada 27: 2009–2036.

VOLLENWEIDER, R. A. 1969. A manual on methods for measuring primary production in aquatic environments. Blackwell Scientific Publications, Oxford, England. 213 p.

Comments on the Nomenclature of Some Subgenera of Salmonidae

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VLADYKOV, V. D., AND C. G. GRUCHY. 1972. Comments on the nomenclature of some subgenera of Salmonidae. J. Fish. Res. Bd. Canada 29: 1631–1632.

Nomenclatural problems in some subgenera of Salmonidae are cleared away with the diagnoses of three new subgenera, viz: *Parahucho* Vladykov, *Parasalmo* Vladykov, and *Rhabdofario* Cope.

VLADYKOV, V. D., AND C. G. GRUCHY. 1972. Comments on the nomenclature of some subgenera of Salmonidae. J. Fish. Res. Bd. Canada 29: 1631–1632.

La diagnose de trois nouveaux sous-genres: *Parahucho* Vladykov, *Parasalmo* Vladykov, et *Rhabdofario* Cope, permet d'éclaircir des problèmes de nomenclature de certains sous-genres de salmonidés.

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IN conjunction with our studies of Salmonidae, we have been aware of subgeneric nomenclatural problems for some time. It is our intention to clear these matters away in the present note.

One of us (Vladykov 1963) earlier proposed two new subgenera of Salmonidae, but neglected to designate type-species at that time. In consequence, the names are unavailable according to Article 42 (b) of the International Code of Zoological Nomenclature (1964). This situation is rectified

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herein, and we wish to emphasize that these two names take the date of issuance of this journal.

Parahucho Vladykov, New Subgenus

Type species designated herewith: *Salmo perryi* Brevoort 1856. Diagnosis: This subgenus is distinguished from the nominate subgenus, *Hucho* Günther 1866, by having 70–100 fewer scales in the lateral line, ten fewer vertebrae, by the presence of basibranchial teeth, and by the median series of teeth on the supralingual.

Parasalmo Vladykov, New Subgenus

Type species designated herewith: *Salmo clarkii*. Richardson 1836. Diagnosis: This subgenus is distinguished from other extant subgenera of *Salmo* (*Salmo* Linnaeus 1758 and *Platysalmo* Behnke 1968) by the presence of black spotting on the caudal fin, by the absence or great reduction of a medial pocket on the premaxillary (hence a very reduced type in spawning males), by a supralingual which is not tapered anteriorly to a narrow tip, with the articulating head of the maxillary raised above the body of that bone, by the lack of a pectoral axillary appendage, by a typical spring (May–June) spawning period, and by endemism in the North Pacific Ocean and its tributaries.

We do not recognise the subgenus *Trutta* Linnaeus 1758 *sensu* Jordan and Evermann (1896) or Jordan et al. (1930) who grouped the Pacific Ocean *Salmo* (*Parasalmo*, herein) with *Salmo trutta* in one subgenus. We believe that the type species of that nominal subgenus of Jordan and associates, *Salmo trutta* Linnaeus 1758, is consubgeneric with the nominate subgenus *Salmo*.

We are also of the opinion that the extinct *Salmo copei* Uyeno and Miller 1963 (formerly *Rhabdofario lacustris* Cope 1870) should be separated in a distinct subgenus, *Rhabdofario* Cope.

Salmo (*Rhabdofario*) *copei* New Combination

Diagnosis: A subgenus of *Salmo* with the premaxillary elongate as in *Oncorhynchus* but with dentition as in *Salmo*, with the supralingual tapered anteriorly to a narrow tip, with the articulating head of the maxillary on the same plane as the body of that bone.

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BEHNKE, R. J. 1968. A new subgenus and species of trout, *Salmo* (*Platysalmo*) *platycephalus*, from south-central Turkey, with comments on the classification of the subfamily Salmoninae. Mitt. Hamburg Zool. Mus. Inst. 66: 1–15.

BREVOORT, J. C. 1856. Notes on some figures of Japanese fish. *In* Commodore M. C. Perry's Expedition to Japan. Vol. I. Washington, D. C. p. 253–256.

COPE, E. D. 1870. On the fishes of a freshwater Tertiary lake in Idaho, discovered by Capt. Clarence King. Proc. Amer. Phil. Soc. 11: 538–547.

GÜNTHER, A. C. L. G. 1866. Catalogue of the fishes of the British Museum. Physostomi. (London) 6: 1–368.

JORDAN, D. S., AND B. W. EVERMANN. 1896. The fishes of North and Middle America. U.S. Nat. Mus. Bull. 47: 1240 p.

JORDAN, D. S., B. W. EVERMANN, AND H. W. CLARK. 1930. Checklist of the fishes of North and Middle America. U.S. Comm. Fish. Rep. Fiscal Year 1928: 1–670.

LINNAEUS, C. 1758. Systema Naturae. 10th ed. 823 p.

RICHARDSON, J. 1836. Fauna Boreali-Americana: III. Fishes. London, 328 p.

UYENO, T., AND R. R. MILLER. 1963. Summary of late Cenozoic freshwater fish records for North America. Occas. Pap. Mus. Zool. Univ. Mich. 631: 1–34.

VLADYKOV, V. D. 1963. A review of salmonid genera and their broad geographical distribution. Trans. Roy. Soc. Can. 3: 459–504.

Incidence of *Vibrio parahaemolyticus* in Shellfish from Eight Canadian Atlantic Sampling Areas

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THOMSON, W. K., AND C. L. THACKER. 1972. Incidence of *Vibrio parahaemolyticus* in shellfish from eight Canadian Atlantic sampling areas. J. Fish. Res. Bd. Canada 29: 1633–1635.

During July to October, 78 cultures of *Vibrio parahaemolyticus*, closely resembling strains isolated from human cases in Japan, were isolated from clams, mussels, and oysters collected from eight Canadian Atlantic areas. Approximately 22% of the shellfish examined yielded this species, about half being detectable by direct plating, and the rest after enrichment before plating.

THOMSON, W. K., AND C. L. THACKER. 1972. Incidence of *Vibrio parahaemolyticus* in shellfish from eight Canadian Atlantic sampling areas. J. Fish. Res. Bd. Canada 29: 1633–1635.

Durant les mois de juillet à octobre, on a prélevé divers échantillons de palourdes, de moules et d'huîtres dans huit endroits différents sur la côte canadienne de l'Atlantique, plus spécifiquement dans la région des Maritimes. Soixante-dix-huit cultures de *Vibrio parahaemolyticus*, présentant des caractéristiques semblables à celles isolées chez l'homme au Japon, ont été isolées chez ces mollusques. Environ 22% des coquillages examinés étaient contaminés par cette bactérie, dont la moitié a pu être décelée par ensemencement sur milieu de gélose et le reste après ensemencement sur milieu enrichi.

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THE present report shows the incidence of *Vibrio parahaemolyticus*, a pathogenic halophilic bacterium, in clams, mussels, and oysters from eight sampling areas in the Canadian Maritime Provinces. In Japan, this species has caused about half of the food poisoning during the summer season (Sakazaki et al. 1963). Although it has been isolated in a number of widely separated areas outside of Japan, including Europe (Hechelmann et al. 1971), Australia (Wallace and Battey 1971), and the United States (Fishbein et al. 1970), it is significant that nearly all food poisoning due to this species has been reported from Japan. The only confirmed outbreak in North America occurred at a picnic in Maryland where 320 persons became ill after consuming crabs contaminated with *V. parahaemolyticus* (Anon. 1971).

Materials and methods — Fresh clams, mussels, and oysters from eight areas (Fig. 1) were collected by the Inspection Branch, Fisheries Service, Canada Department of the Environment, at 2-week intervals from July to October. These were shipped in plastic bags in ice to be examined for *V. parahaemolyticus*. Five reference strains of *V. parahaemolyticus* were received from Dr H. Iida, Sapporo, Japan; and 10 strains, isolated from human cases of enteritis, were received from Dr H. Zen-Yoji, Tokyo, Japan.

Each sample was homogenized in the proportion of 10-g shellfish meat to 90 ml of 3% NaCl–peptone broth. Aliquots of 0.1 ml from each homogenate were plated directly on two selective media described by Thatcher and Clark (1968): bromothymol blue teepol agar (BTB

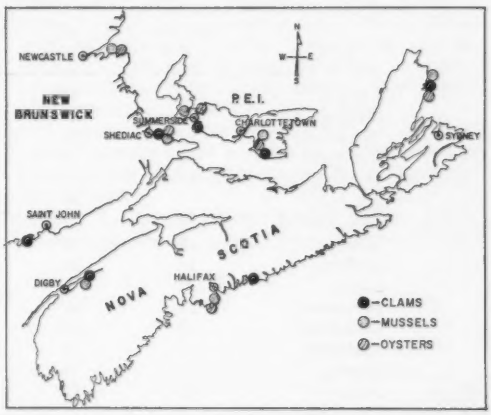

FIG. 1. Locations of eight areas where shellfish were collected.

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Teepol) and thiosulphate citrate bile salts agar (TCBS). Five milliliters of each homogenate was also added to 10-ml bismuth sulphite enrichment broth and incubated at 35 C for 16 hr; 0.1-ml aliquots of the resulting cultures were plated on BTB Teepol and TCBS agar.

The plates were incubated at 42 C for 16 hr in order to inhibit the growth of most halophiles other than *V. parahaemolyticus* (Hechelmann et al. 1971). Colonies suspected of being *V. parahaemolyticus* were picked from plates, inoculated into 3% NaCl–peptone broth, and incubated. All isolates were subjected to determinative tests including microscopic examination, pathogenicity tests, and antigenic analysis; as described previously (Thomson and Trenholm 1971). In addition, the Kanagawa reaction (LeClair et al. 1970) was carried out.

TABLE 1. Numbers of shellfish of the three species from sampled areas yielding *Vibrio parahaemolyticus*.

Sampled area	Date	Clam	Mussel	Oyster
Charlottetown	13 July	1/9[a]	0/8	2/8
Halifax	29 July	4/8	3/8	5/8
Saint John	10 Aug.	2/18	–/–	–/–
Sydney	25 Aug.	1/18	4/6	8/10
Shediac	9 Sept.	2/8	0/8	2/8
Summerside	21 Sept.	1/9	3/4	2/10
Newcastle	7 Oct.	–/–	0/12	2/12
Digby	20 Oct.	1/12	1/11	–/–
Total[b]		12/82	11/57	21/56

[a]No. positive/No. examined.

[b]Grand total: $\frac{44}{195}$, or 22.6%.

Results — Forty-four out of 195 (22%) of the shellfish collected yielded *V. parahaemolyticus* (Table 1) and positive samples were found in all areas. Approximately one-half of the samples required the enrichment procedure to facilitate detection of small numbers of cells.

Seventy-eight isolates of *V. parahaemolyticus* were obtained from the shellfish. Of the 30 tests of these isolates, 23 agreed 100% with those of 15 Japanese reference strains (Table 2). The remaining seven tests showed some variation, but fell within limits that have been reported for the species by other workers. We found that 32 strains did not ferment arabinose (Sakazaki et al. 1968), 23 strains fermented cellobiose (Twedt et al. 1969; Colwell 1970), 9 strains did not liquify gelatin (Iida 1967), 3 strains were non-haemolytic on 3% NaCl–human blood agar, and 22 strains were non-haemolytic on Wagatsuma agar (Kanagawa phenomenon) (Sakazaki et al. 1968; Miyamoto et al. 1969; Hechelmann et al. 1971). Twenty-three of the isolates were non-pathogenic for mice by the intraperitoneal route (Sakazaki et al. 1963). About 40% of our isolates were shown to contain K antigens, of which K17, K20, K24, K33, K34, and K39 occurred more than once.

Discussion — The survey indicates a wide distribution (Fig. 1) of shellfish contaminated with *V. parahaemolyticus*. The 22% incidence of *V. parahaemolyticus* in Canadian east-coast shellfish is approximately the same as the 28% reported in European samples by Hechelmann et al. (1971).

TABLE 2. Numbers of the isolates (total 78) and of the Japanese reference strains (total 15) that gave positive reactions for *Vibrio parahaemolyticus* in determinative tests.[a]

Test	Japanese strains	Canadian isolates	Test	Japanese strains	Canadian isolates
Lactose	0	0	Gelatin	15	69[b]
Sucrose	0	0	Motility	15	78
Mannitol	15	78	H_2S	0	0
0% NaCl	0	0	Tartrate	15	78
3% NaCl	15	78	Indole	15	78
7% NaCl	15	78	Blood Agar	15	75[b]
10% NaCl	0	0	Kanagawa	15	56[b]
Glucose	15	78	Catalase	15	78
Maltose	15	78	Cytochrome Oxidase	15	78
Salicin	0	0	Voges-Proskauer	0	0
Arabinose	12	46[b]	Nitrite produced	15	78
Cellobiose	0	23[b]	Mouse pathogen	15	55[b]
Adonitol	0	0	Growth on BTB	15	78
Inositol	0	0	Growth on TCBS	15	78
Starch	15	78	K Antigen	15	32[b]

[a]Details of tests for each strain available on request to authors.

[b]*V. parahaemolyticus* tests reported to be variable.

Forty-one percent of the Canadian isolates possessed K antigens, in comparison with 40% of the isolates of Fishbein et al. (1970) in the eastern United States and 48% of those of Hechelmann et al. (1971) in Europe. Serotypes K_{17}–K_{39} were predominant in these areas.

The majority of our isolates appear to be capable of causing food-borne infection. Sakazaki et al. observed that *V. parahaemolyticus* strains isolated from persons suffering from gastro-enteritis are Kanagawa-positive (1968) and pathogenic for mice (1963). In the present investigation, 56 out of the 78 Canadian strains were Kanagawa-positive and 55 of these were also pathogenic for mice.

The numbers of *V. parahaemolyticus* in our samples were small as indicated by approximately half the samples requiring enrichment before the bacteria could be detected. Food poisoning resulting from the consumption of such small numbers would appear to be unlikely, even if the shellfish were consumed raw. Smith (1971) reported that enteritis in man requires the ingestion of 10^6–10^9 bacteria.

However, the possibility of human disease in Canada cannot be completely dismissed. Large numbers of *V. parahaemolyticus* can develop if shellfish are not properly refrigerated during transportation and during storage at the retailer's or in the home. Bauman (1972) reported refrigerated showcases to cycle between 4.4 C and 12.8 C over a 24 hr period, and van Walbeek et al. (1969) observed that, during a survey of domestic refrigerators, temperatures were measured up to 10 C. W. K. Thomson and C. L. Thacker (unpublished data) have observed that *V. parahaemolyticus* multiplies at temperatures as low as 10 C.

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ANON. 1971. *Vibrio parahaemolyticus* gastroenteritis—Maryland. Morbidity Mortality Wkly. Rep. 20: 356.

BAUMAN, H. E. 1972. Food microbiology. Amer. Soc. Microbiol. News 38: 312–317.

COLWELL, R. R. 1970. Polyphasic taxonomy of the genus Vibrio: numerical taxonomy of *Vibrio cholerae*, *Vibrio parahaemolyticus* and related Vibrio species. J. Bacteriol. 104: 410–433.

FISHBEIN, M., I. J. MEHLMAN, AND J. PITCHER. 1970. Isolation of *Vibrio parahaemolyticus* from processed meat of Chesapeake Bay blue crabs. Appl. Microbiol. 20: 176–178.

HECHELMANN, H., K. TAMURA, T. INAL, AND L. LEISTNER. 1971. Vorkommen von *Vibrio parahaemolyticus* in verschiedenen Seegebieten Europas im Jahre 1970. Die Fleischwirtsch. Heft Seite 6: 965–968.

IIDA, H. 1967. Food poisoning caused by *Vibrio parahaemolyticus*. Rep. Hokkaido Inst. Pub. Health 11: 209–212.

LECLAIR, R. A., H. ZEN-YOJI, AND S. SAKAI. 1970. Isolation and identification of *Vibrio parahaemolyticus* from clinical specimens. Pub. Health Lab. 28: 82–92.

MIYAMOTO, Y., T. KATO, Y. OBARA, S. AKIYAMA, K. TAKIZAWA, AND S. YAMAIS. 1969. *In vitro* haemolytic characteristic of *Vibrio parahaemolyticus*: its close correlation with human pathogenicity. J. Bacteriol. 100: 1147–1149.

SAKAZAKI, R., S. IWANAMI, AND K. TAMURA. 1968. Studies on the enteropathogenic, facultatively halophilic bacteria, *Vibrio parahaemolyticus*. II. Serological characteristics. Jap. J. Med. Sci. Biol. 21: 313–324.

SAKAZAKI, R., I. SETSUO, AND H. FUKUMI. 1963. Studies on the enteropathogenic, facultatively halophilic bacteria, *Vibrio parahaemolyticus*. I. Morphological, cultural and biochemical properties and its taxonomical position. Jap. J. Med. Sci. Biol. 16: 161–188.

SMITH, M. R. 1971. *Vibrio parahaemolyticus*. Clin. Med. 78: 22–25.

THATCHER, F. S., AND D. S. CLARK. 1968. Their significance and methods of enumeration, p. 107–114. *In* Microorganisms in food. University of Toronto Press, Toronto, Ont.

THOMSON, W. K., AND D. A. TRENHOLM. 1971. The isolation of *Vibrio parahaemolyticus* and related halophilic bacteria from Canadian Atlantic shellfish. Can. J. Microbiol. 17: 545–549.

TWEDT, R. M., P. L. SPAULDING, AND H. E. HALL. 1969. Morphological, cultural, biochemical and serological comparison of Japanese strains of *Vibrio parahaemolyticus* with related cultures isolated in the United States. J. Bacteriol. 98: 511–518.

VAN WALBEEK, W., T. CLADEMENOS, AND F. S. THATCHER. 1969. Influence of refrigeration on aflatoxin production by strains of *Aspergillus flavus*. Can. J. Microbiol. 15: 629–632.

WALLACE, R. B., AND Y. M. BATTEY. 1971. *Vibrio parahaemolyticus* in oysters. Med. J. Aust. 2: 982.

Use of the DeLury Method to Estimate Gillnet Selectivity[1]

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HAMLEY, J. M. 1972. Use of the DeLury method to estimate gillnet selectivity. J. Fish. Res. Bd. Canada 29: 1636–1638.

Catch–effort methods of population estimation can also be applied to estimate the selectivity of fishing gear. Extensions of DeLury's formulae are considered, and the method is demonstrated on published data of gillnetting whitefish.

HAMLEY, J. M. 1972. Use of the DeLury method to estimate gillnet selectivity. J. Fish. Res. Bd. Canada 29: 1636–1638.

Les méthodes capture-effort utilisées dans l'estimation des populations de poissons peuvent également s'appliquer aux calculs de sélectivité d'engins de pêche. Nous considérons certains corollaires des équations de DeLury et démontrons la validité de la méthode en l'appliquant à des données sur la pêche du grand corégone de lac aux filets maillants.

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LESLIE and Davis (1939) and DeLury (1947) developed a technique for estimating catchability and population size from catch–effort data. When a closed population is fished, catches usually decline as this population decreases. Under certain assumptions this decline can be represented by a linear regression of catch per unit effort against the accumulated catch or the accumulated effort. Catchability and initial population can then be estimated from the slope and the y intercept of the regression line. This technique can be extended to estimate the selectivity of the sampling gear.

Theory — If, during any short time interval t, the catch C_t is proportional to both the fishing effort X_t and the current population size N_t, the expected catch per effort $(C/X)_t$ for the tth interval is

$$(C/X)_t = k_t N_t \tag{1}$$

where k_t is a constant that defines the catchability during t. N_t can also be expressed in terms of the original population N_o and the total catch $\sum_t C_t$ accumulated to time t:

$$N_t = N_o - \sum_t C_t. \tag{2}$$

If $k_t = k$ for all t, then for any t

$$(C/X)_t = kN_o - k\sum_t C_t. \tag{3}$$

DeLury (1947) shows that this implies

$$\ln(C/X)_t = \ln(kN_o) - k\sum_t X_t \tag{4}$$

where $\sum_t X_t$ is the total accumulated fishing effort. The summations $\sum_t C_t$ and $\sum_t X_t$ can be accumulated either to the beginning or to the end of the tth time period (DeLury 1947), or they may include half of the catch or effort of the tth time period (Braaten 1969).

Initial population and catchability can be estimated from either (3) or (4). If catchability remains constant, a plot of $(C/X)_t$ against $\sum_t C_t$ approximates a straight line with slope $-k$ and y intercept kN_o. Similarly, a plot of $\ln(C/X)_t$ against $\sum_t X_t$ approximates a straight line with slope $-k$ and y intercept $\ln(kN_o)$. The values of k and N_o are estimated by linear regression of one of these plots. However, (4) may give biased estimates, since in fact $\sum_t X_t$ represents mortality but does not estimate it without error as assumed in regressing $\ln(C/X)_t$ against $\sum_t X_t$ (J. E. Paloheimo personal communication). The statistical confidence intervals for (3) are in DeLury (1951) and, for (4), in Nose (1959).

The selectivity s_j of the gear can be estimated by treating each size class j of fish separately:

$$(C_j/X)_t = s_j N_{jo} - s_j \sum_t C_{jt} \tag{5}$$

$$\ln(C_j/X)_t = \ln(s_j N_{jo}) - s_j \sum_t X_t \tag{6}$$

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[1]Based in part on a thesis submitted to the University of Toronto in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Selectivity should be interpreted as the probability of capture, given the fishing effort — not as the probability of capture after a fish has encountered the net. Under the latter interpretation, $s_j N_j$ would not describe the catches nor would X_t represent the effective effort for all j, since the probability of encounter may vary with fish size.

If several sizes of mesh are fished simultaneously, their selectivities can be estimated if, during any time interval t, the catch by any one mesh is independent of the effort by the others. Then, (5) and (6) can be applied separately to each mesh size i:

$$(C_{ij}/X_i)_t = s_{ij}N_{jo} - s_{ij}\sum_t\sum_t C_{ijt} \tag{7}$$

$$\ln(C_{ij}/X_i)_t = \ln(s_{ij}N_{jo}) - \sum_t(s_{ij}\sum_t X_{it}) \tag{8}$$

Since the accumulated effort in (6) represents the accumulated mortality, the last term in (8) must account for the total mortality of fish of size j, caused by all mesh sizes. Equation (7) can estimate s_{ij} as before, but equation (8) cannot because it involves s_{ij} for all i.

The catches by different mesh are proportional to the selectivities of these mesh (Holt 1963)

$$\frac{\sum_t C_{ijt}}{\sum_i\sum_t C_{ijt}} = \frac{s_{ij}}{\sum_i s_{ij}} \tag{9}$$

This allows the estimation of selectivities s_{ij} of each mesh even if periodic records exist of only the combined catches by all mesh sizes, as long as the total catches accumulated by each mesh are known. The selectivity of the entire set of gear is first estimated by (5) or (6), then apportioned to the individual mesh sizes according to (9).

Example — The method may be demonstrated with Hart's (1932) data on gillnetting whitefish from Shakespeare Island Lake, Ontario. He made seven series of sets in that lake, using a gang of 11 gillnets of different mesh sizes. During this study catches declined by more than a half. Assuming this decline to be proportional to the decline in the population, Ricker (1947: Table 1, column b) calculated regression coefficients which correspond to s_j in (6). (Actually, they correspond to $(\log_{10}e)s_j = 0.4343s_j$, since Ricker used common rather than natural logarithms.) These coefficients represent the selectivity of the entire gang of nets to these fish (Fig. 1).

The selectivities of individual mesh can be estimated if each mesh is assumed to have fished independently. Equation (7) cannot be used since Hart did not report periodic catches separately for each mesh size. However, (9) can be used to apportion Ricker's total selectivity estimates among the mesh sizes. Figure 2 shows the resulting selectivity curves,

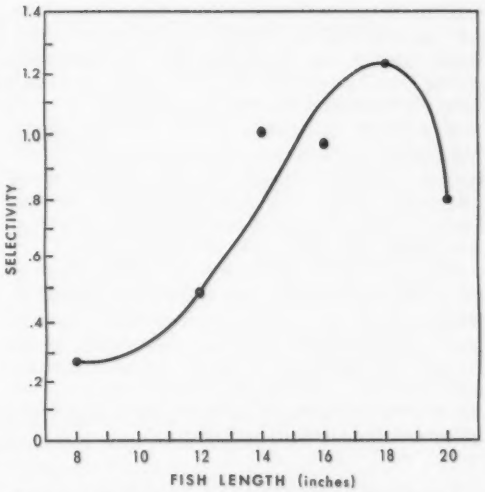

FIG. 1. Selectivity of a mixed gang of gillnets to whitefish (data from Ricker 1947).

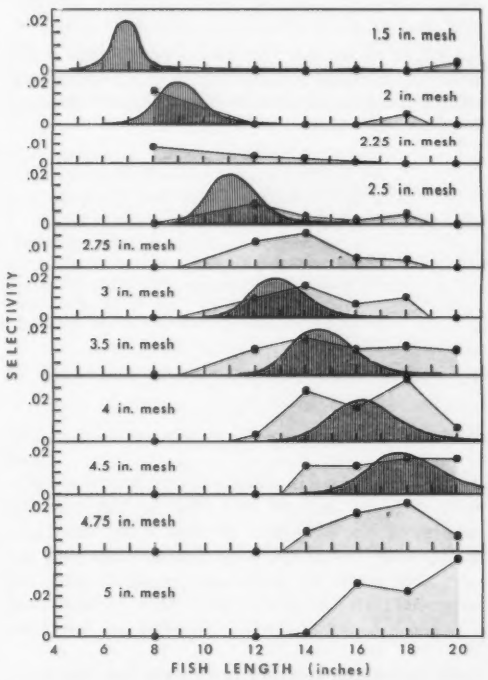

FIG. 2. Selectivity of 1½ to 5-inch gillnets to whitefish. Lines enclosing dotted areas estimated by DeLury method from data of Hart (1932); smooth curves (lined areas) estimated by Regier and Robson (1966).

and the corresponding selectivity curves for whitefish as estimated by Regier and Robson (1966). Regier and Robson assumed that the selectivity curves for all mesh have the same height, and may have overestimated the relative selectivity of small mesh sizes (H. A. Regier personal communication). The DeLury method makes no such assumption and estimates the relative heights as well as shapes of the selectivity curves. The heights estimated by the DeLury method increased with mesh size, indicating that larger-mesh nets were more efficient.

Although Hart's study was not intended as a selectivity study and his nets differed from those of Regier and Robson, the two sets of selectivity estimates agree enough to support the validity of the DeLury estimates. Hart's use of 11 mesh sizes resulted in highly variable selectivity estimates. Also, the surrounding nets of different mesh sizes likely influenced the catch by any one net.

Conclusion — The DeLury method deserves more attention in the estimation of gear selectivity. Its advantages are that it requires no independent population estimates, no comparison between different gear, and no prior assumptions about the nature of the selectivity curves. The main disadvantage is the difficulty of experimentally satisfying its underlying assumptions, especially that the selectivity remains constant with time.

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BRAATEN, D. O. 1969. Robustness of the DeLury population estimator. J. Fish. Res. Bd. Canada 26: 339–355.

DELURY, D. B. 1947. On the estimation of biological populations. Biometrics 3: 145–167.

1951. On the planning of experiments for the estimation of fish populations. J. Fish. Res. Bd. Canada 8: 281–307.

HART, J. L. 1932. Statistics of the whitefish (*Coregonus clupeaformis*) population of Shakespeare Island Lake, Ontario. Univ. Toronto Stud., Biol. Ser. 36, Publ. Ont. Fish. Res. Lab. 42: 1–28.

HOLT, S. J. 1963. A method for determining gear selectivity and its application. Int. Comm. Northwest Atl. Fish. Spec. Publ. 5: 106–115.

LESLIE, P. H., AND D. H. S. DAVIS. 1939. An attempt to determine the absolute number of rats on a given area. J. Anim. Ecol. 8: 94–113.

NOSE, Y. 1959. On the confidence limits corresponding to the estimate obtained by DeLury's logarithmic catch-effort method. Bull. Jap. Soc. Sci. Fish. 24: 953–956.

REGIER, H. A., AND D. S. ROBSON. 1966. Selectivity of gill nets, especially to lake whitefish. J. Fish. Res. Bd. Canada 23: 423–454.

RICKER, W. E. 1947. Mortality rates in some little-exploited populations of fresh-water fishes. Trans. Amer. Fish. Soc. 77: 114–128.

Occurrence of *Glugea hertwigi* in Lake Erie Rainbow Smelt (*Osmerus mordax*) and Associated Mortality of Adult Smelt[1]

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NEPSZY, S. J., AND A. O. DECHTIAR. 1972. Occurrence of *Glugea hertwigi* in Lake Erie rainbow smelt (*Osmerus mordax*) and associated mortality of adult smelt. J. Fish. Res. Bd. Canada 29: 1639–1641.

The occurrence of cysts of *Glugea hertwigi*, a microsporidian parasite, in Lake Erie rainbow smelt (*Osmerus mordax*) has been monitored since 1960 when it was first recorded. Annual sampling has shown that an increasing proportion (up to 87.5% in 1971) of the smelt population has been infected.

An unusually severe mortality among adult smelt occurred during early May, 1971, in western and west central Lake Erie. Sampling of dead and obviously stressed smelt revealed that 97.3% of the males and 76.7% of the females were heavily loaded with cysts in the intestines and gonads, respectively. It is suggested that the parasite load contributed to the unusual severity of the normal postspawning mortality.

NEPSZY, S. J., AND A. O. DECHTIAR. 1972. Occurrence of *Glugea hertwigi* in Lake Erie rainbow smelt (*Osmerus mordax*) and associated mortality of adult smelt. J. Fish. Res. Bd. Canada 29: 1639–1641.

L'apparition de kystes de *Glugea hertwigi*, une microsporidie parasite de l'éperlan arc-en-ciel (*Osmerus mordax*) du lac Erié, a été suivie de près depuis son premier signalement en 1960. L'échantillonnage annuel démontre qu'une proportion croissante (atteignant 87.5% en 1971) de la population est infestée.

Des mortalités extrêmement sévères se sont produites chez les éperlans adultes des secteurs ouest et central-ouest du lac Erié au début de mai, 1971. L'examen de poissons morts ou manifestement malades a révélé que 97.3% des mâles et 76.7% des femelles avaient un nombre considérable de kystes, respectivement dans l'intestin et dans les gonades. Cette infestation aurait contribué, d'après les auteurs, à élever à un niveau catastrophique les mortalités qui suivent normalement la fraie.

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MORTALITIES of adult rainbow smelt (*Osmerus mordax*) during the period immediately after spawning have been observed for several years along the shorelines of Lake Erie. These mortalities have been attributed to exhaustion after spawning and have been looked upon as normal. The mortality which occurred in early May, 1971, appeared to be unusually severe in the western basin and the western portion of the central basin and because of its severity a special study was made of the dead fish.

The microsporidian parasite *Glugea hertwigi* was first recorded in Lake Erie rainbow smelt in 1960 (Dechtiar 1965) and its frequency of occurrence has been documented since this date. This parasite has been reported elsewhere as contributing to mortalities of smelt (Haley 1952) and stint (*Osmerus eperlanus* m. *spirinchus* (Bogdanova 1957)).

Methods and materials — Random samples of smelt have been taken monthly since 1964 from commercial trawlers fishing in the eastern and central basins of Lake Erie. Samples were not obtained routinely from the western basin since the trawl fishery does not operate

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there. Fish were examined internally and externally for the frequency of occurrence of *G. hertwigi* cysts. Cysts were classified as small (<1-mm diameter), medium (1–2-mm), or large (> 2-mm); and the frequency of their occurrence was recorded.

The shoreline survey following severe mortality in early May extended from Amherstburg to Port Dover. Accumulations of dead smelt were found only on the beaches from Amherstburg to Erieau. Samples of dead and apparently stressed smelt were collected from several areas on May 11, 12, and 18. Numbers of dead smelt per square meter of shore area were counted at additional sampling sites. A total of 161 males and 75 females were examined in detail.

Results — The incidence of *G. hertwigi* cysts has been increasing since its occurrence was first noted in 1960 (Table 1). Smelt from the central basin have generally shown a greater percentage occurrence of cysts. The presence of *G. hertwigi* cysts in smelt involved in this study was extremely high.

The average number of dead smelt per square meter of shoreline, based on counts of 23 plots, was 35; and the length of the shoreline affected by this mortality was 120 km. Ignoring smelt on the lake bottom or floating offshore, minimal mortality was estimated at 4,235,000 (about 70.6 metric tons or 1% of Canadian smelt landings in 1969).

Most fish sampled were 2-year-olds (1969 year-class) with an average total length of 15 cm. Studies of spawning populations in the Wheatley area during April, 1971, indicated that most spawners were also 2-year-old fish. Gillnet catches off Wheatley during the spawning run showed that the greatest abundance of male smelt occurred on approximately April 22 while females outnumbered males on April 29. Therefore, fish involved in this mortality probably spawned 10 days earlier.

Of the 236 fish examined, 148 males and 73 females were infected with *G. hertwigi* cysts. The greatest percentage occurrence of cysts was found on the intestines of both males and females, 97 and 79%, respectively (Fig. 1). Percentage occurrences in the other organs of the males were: gonads, 17%; stomach, 0.7%; mesentery, 1.4%; muscle, 0.7%; and liver, 1.4%. In the females the other organs affected were the gonads and the stomach with 77 and 1.4% occurrence, respectively. Cyst diameters ranged from 0.2 to 5 mm. All cyst sizes were found on the intestines and in the gonads of both sexes. However, the greatest frequency was evident on the intestines of males where large cysts predominated. Large cysts also predominated in the female ovaries but not to the extent found on the male intestines.

Discussion — While most of the dead smelt were age 2 (1969 year-class) and seriously parasitized by *G. hertwigi*, it is noteworthy that smelt of that year class have been heavily infected throughout their life. Samples (from index fishing) taken at age 0+ in 1969 showed 88.8% to be infected. In October, 1969, in the east central and eastern basins of Lake Erie there was a mortality of young-of-the-year smelt, the details of which are still being investigated. This mortality may have been caused by heavy *G. hertwigi* parasitization. In 1970, at age 1+, over 90% of the 1969 year-class sampled were infected. These data suggest that the parasite enters the fish at an early age and may remain throughout the life of the host. The stress imposed by a heavy infestation may be such that the normal stresses of environmental changes or physiological activities, such as spawning, become critical in the survival of the host.

Recorded mortalities of adult smelt in the past have been attributed to postspawning exhaustion, to infectious disease (VanOosten 1947), and to consequences of infection by *G. hertwigi* (Haley 1952; Bogdanova 1957). Haley (1954) suggested that the pathological consequences of *G. hertwigi* infection were an important contributing factor to the smelt decline in the Great Bay region of

TABLE 1. Percentage occurrence of *Glugea hertwigi* cysts in smelt examined from the eastern and central basins of Lake Erie from 1960 to 1971.[1]

Location	Year and percentage occurrence							
	1964	65	66	67	68	69	70	71
Eastern basin	4.6	9.1	31.2	42.1	58.9	73.4	85.8	87.9
(No. of fish examined)	4987	2467	1898	2855	4559	1527	2021	3323
Central basin	11.7	23.0	35.9	51.1	61.2	74.0	80.1	87.2
(No. of fish examined)	5301	2822	1578	1503	3206	884	1556	1012

[1]Data for eastern basin: 1960, 12.5(16); 1961, 13.3(15); 1963, 15.0(20). No data available for eastern basin, 1962; or central basin, 1960–63, inclusive.

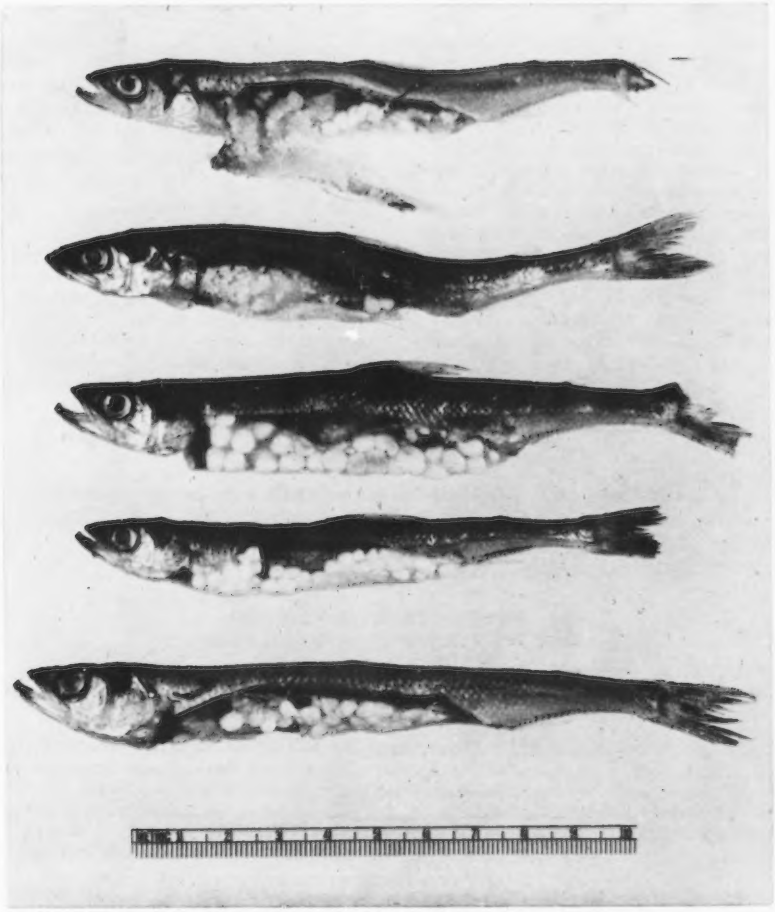

FIG. 1. Smelt in spent condition showing organs and tissues infected by cysts of the microsporidian *Glugea hertwigi*.

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New Hampshire. Legault and Delisle (1967) and Haley (1952) concluded that death could have resulted from starvation or intestinal poisoning associated with disintegration of the intestinal tissues. Cases of massive infestation by the parasite caused mortalities of the stint (*O. eperlanus* m. *spirinchus*) and produced degenerative changes in the sexual organs (Markevich 1951; Bogdanova 1957).

The proportion of the smelt population infected by *G. hertwigi* has been increasing and the cyst load per host has become progressively heavier. It is our opinion that the stress imposed by this parasite load on the 1969 year-class of smelt was such that it escalated the normal postspawning mortality.

Acknowledgments — The authors thank Mr K. H. Loftus for his valuable criticisms and assistance in reviewing the manuscript. Dr J. Leach also critically read the manuscript and offered helpful suggestions. We acknowledge the assistance of Messrs L. Drummond, J. Kippax, J. Murphy, and A. Matthews in sampling and in preparing the data.

Bogdanova, E. A. 1957. The myxosporidan, *Glugea hertwigi* Weissenberg, in the stint (*Osmerus eperlanus* m. *spirinchus*) from Yulya-Yarvi. Bull. All-Union Sci. Res. Inst. Freshwater Fish. 42: 328.

Dechtiar, A. O. 1965. Preliminary observations on *Glugea hertwigi* Weissenberg, 1911 (Microsporidia; Glugeidae) in American smelt, *Osmerus mordax* (Mitchill) from Lake Erie. Can. Fish Cult. 34: 35–38.

Haley, A. J. 1952. Preliminary observations on a severe epidemic of microsporidosis in the smelt, *Osmerus mordax* (Mitchill). J. Parasitol. 38: 183.

1954. Microsporidian parasite, *Glugea hertwigi*, in American smelt from the Great Bay region, New Hampshire. Trans. Amer. Fish. Soc. 83: 84–90.

Legault, R. O., and C. Delisle. 1967. Acute infection by *Glugea hertwigi* Weissenberg in young-of-the-year rainbow smelt, *Osmerus eperlanus mordax* (Mitchill). Can. J. Zool. 45: 1291–1292.

Markevich, A. P. 1951. Parasitic fauna of freshwater fish of the Ukrainian S.S.R., p. 82–84. (Transl. from Russian. U.S. Dep. Inter.)

VanOosten, J. 1947. Mortality of smelt, *Osmerus mordax* Mitchill, in Lakes Huron and Michigan during the fall and winter of 1942–43. Trans. Amer. Fish. Soc. 74: 310–337.

Comparison of the Efficiency of Various Aeration Devices for Oxygenation of Water in Aquaria

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Scott, K. R. 1972. Comparison of the efficiency of various aeration devices for oxygenation of water in aquaria. J. Fish. Res. Bd. Canada 29: 1641–1643.

Efficiencies of aeration of five different combinations of venturis, air stones, and sprays were measured in a 1.8-m³ tank. Results showed that the venturi method discharging through a nozzle near the tank bottom had the highest efficiency. Air stones were 53% as effective and sprays directed at the surface 41% as effective as the venturi method. Details are given for fabrication of the PVC (polyvinyl chloride) venturi. The unit is reliable, inexpensive, and eliminates the need for a separate air supply.

Scott, K. R. 1972. Comparison of the efficiency of various aeration devices for oxygenation of water in aquaria. J. Fish. Res. Bd. Canada 29: 1641–1643.

L'auteur compare l'efficacité des venturis, des pierres poreuses, et des jets pulvérisés, utilisés en cinq combinaisons différentes, pour l'aération de l'eau dans un bassin de 1800 litres. Il constate que le venturi aboutissant à une busette près du fond du bassin est le plus efficace. La pierre poreuse et le jet dirigé vers la surface ont une efficacité de 53% et 41%, respectivement, de celle du venturi. Il explique en détail la fabrication du venturi avec du chlorure de polyvinyle (CVP). L'appareil est sûr, peu dispendieux, et supprime le besoin d'un approvisionnement d'air séparé.

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Oxygen concentration in aquaria is an important parameter in maintaining a healthy environment for rearing and maintaining fish. Several different techniques for increasing oxygen level in fish holding tanks have been used; including agitators, sprays, carborundum air stones or perforated tubes using compressed air, and venturi inspirators. The most commonly used methods are sprays, air stones, or venturis. The purpose of this study was to evaluate the relative efficiencies of these three methods separately and in combination.

Theory — Since oxygenation is dependent on a diffusion mechanism, the rate of change of oxygen concentration at any time "t" is proportional to the difference between the saturation concentration C_s and the existing concentration C with the proportionality constant being a measure of the effectiveness of the particular aeration device being used. In equation form:

$$\frac{dC}{dt} = K\,(C_s - C) \quad \ldots\ldots(1)$$

The solution to this differential equation is

$$C = C_s\,(1 - e^{-K(t-t_o)}) \quad \ldots\ldots(2)$$

Rearranging and taking the natural log of both sides yields

$$ln\,(1 - C/C_s) = -K(t - t_o) \quad \ldots\ldots(3)$$

A plot of $ln\,(1-C/C_s)$ vs time should yield a straight line with slope K, having t_o equal to the value of t where the line intersects the ordinate $1-C/C_s = 1.0$. The closeness of the fit of the experimental points to this line will indicate the accuracy of the initial assumption regarding relationship of the variables.

Materials and methods — Tests were carried out in a 1.8-m³ cylindrical tank 1.83 m wide and 0.76 m high. Water circulation rate was held at 950 cm³/sec. The surface of the water was covered by a 4-mil polyethylene sheet to exclude air which would otherwise be redissolved at the surface of the water. Nitrogen was bubbled into the water at a rate of 511 cm³/sec via two air stones. When the O_2 concentrations in the water reached ap-

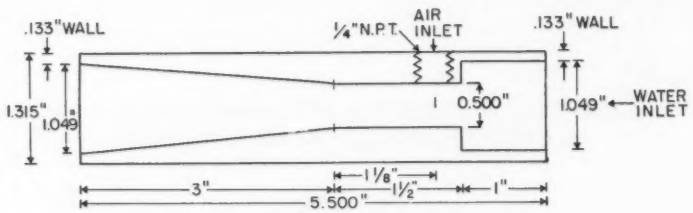

Fig. 1. Polyvinyl chloride venturi inspirator.

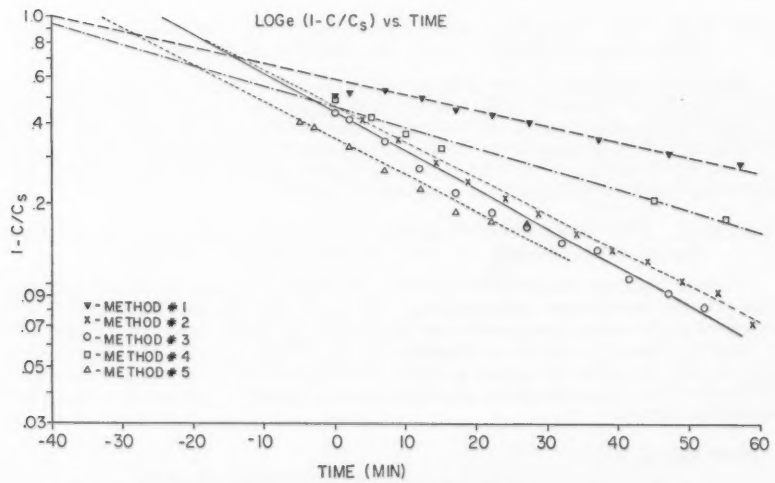

Fig. 2. Plot of log e $(1-C/C_s)$ vs. time for five different aeration methods.

proximately 50% of saturation values; the nitrogen was turned off, the polyethylene cover removed, and the aeration device under test was turned on. Measurements of oxygen concentration and temperature were taken at 5-min intervals until the changes became minimal due to the asymptotic approach to saturation values.

Five different methods of aeration were tested: 1) No venturi with water spraying at a 30° angle from a normal to the water surface via a horizontal header drilled with small holes to promote air entrainment by the resulting high velocity jet stream; 2) Venturi installed in vertical pipe with discharge pipe going down 8 inches below the water surface, turning 90°, and discharging through a 1.9 cm diameter nozzle tangential to the tank wall; 3) Same as 2 except discharging near the bottom of the tank; 4) Two 12-inch long air stones on the bottom of the tank with the air flow adjusted to 550 cm^3/sec, the same rate that air was inspirated by the venturi; 5) Combination of methods 3 and 4, i.e., venturi discharging near the bottom through a nozzle plus two air stones.

The venturi (Fig. 1) was machined from PVC (polyvinyl chloride) and designed to fit directly into a 1-inch IPS socket coupling giving full flow operation with minimal pressure loss. Air was drawn in through a ¼-inch NPT tapped opening at the *vena contracta* of the venturi. The air became intimately mixed with the water and was then discharged into the tank via the nozzle. Water was circulated with a ½ hp 3450 rpm continuous duty motor close-coupled to a centrifugal pump having a Buna-N body and impeller and a capacity of 1583 cm^3/sec at a 7.6-m water head.

Oxygen concentration readings were taken with a YSI Model 51 meter and corrected for station altitude and temperature.

Results and discussion — After removal of the cover and activation of the various aeration devices, the oxygen concentration rose to approach saturation levels asymptotically. A plot of $(1-C/C_s)$ vs t on semi-log paper (Fig. 2) shows that, for all devices tested, the experimental points fell approximately on straight lines of different slopes. This confirms the validity of the original assumption.

Values of the slope K for each of the five aeration methods were:

Method	K (ppm(wt basis)/min/unit concn. gradient)
1	.0135
2	.0310
3	.0331
4	.0176
5	.0314

Except for method 1 (sprays only), there was no measurable difference in oxygen concentration between the top and the bottom of the tank. For method 1 the values were approximately 0.2 ppm lower at the bottom than the top indicating poorer mixing for this method.

It is necessary to have a restricting nozzle in the discharge pipe when using a venturi discharging below the surface in order to promote the formation of a water-air mixture having very small bubbles and a long jet stream. This enhances efficient mixing, resulting in more rapid diffusion of the air into the water.

The amount of restriction that can be added downstream of the venturi depends on the head loss in the pipe, depth below the surface, and the water flow. Too much restriction for any particular configuration will result in a decrease in aeration.

The text table above shows that a venturi inspirator discharging through a nozzle near the bottom of the tank gives the best rate of oxygenation producing rates almost twice as high as air stones. The next best method is also the venturi but discharging close to the surface. Sprays directed at the water surface were the least effective and the addition of air stones to the venturi system did not result in any increase in effectiveness, the results actually indicating a slight decrease which is not significant.

In addition to a much higher operating efficiency, a venturi does not require a separate compressed air subsystem for operation and, hence, is more reliable. This is an important consideration in fish holding facilities since air failure, particularly at high loading densities, can quickly result in high mortality rates. The cost of $8.50 including material and machining is competitive with alternate methods.

Mercury in Harbour Porpoises (*Phocoena phocoena*) From the Bay of Fundy Region

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GASKIN, D. E., K. ISHIDA, AND R. FRANK. 1972. Mercury in harbour porpoises (*Phocoena phocoena*) from the Bay of Fundy region. J. Fish. Res. Bd. Canada 29: 1644–1646.

During 1969–71, 41 muscle and 20 liver samples were collected from harbour porpoises (*Phocoena phocoena*) in the Bay of Fundy region. These tissues were analyzed for total mercury content. Total Hg levels ranged from 0.21 to 1.92 ppm (average 0.75) in muscle tissue of males and from 0.26 to 2.58 ppm(average 1.02) in muscle tissue of females; from 0.89 to 18.30 ppm in liver tissue of males and from 0.55 to 91.30 ppm in liver tissue of females. Averages for the two latter series would be meaningless. A limited number (four muscle and six liver samples) of determinations of the methylated fraction were also made. In the muscle, Hg was virtually 100% methylated; in the liver the methylated fraction varied from 7.4 to 41%, being lowest in livers with highest total Hg.

GASKIN, D. E., K. ISHIDA, AND R. FRANK. 1972. Mercury in harbour porpoises (*Phocoena phocoena*) from the Bay of Fundy region. J. Fish. Res. Bd. Canada 29: 1644–1646.

De 1969 à 1971, les auteurs ont recueilli 41 échantillons de muscle et 20 de foie de marsouin commun (*Phocoena phocoena*) dans la région de la baie de Fundy. Ils ont déterminé la teneur en mercure total de ces tissus. La teneur en Hg total du muscle varie de 0.21 à 1.92 ppm (moyenne 0.75) chez les mâles et de 0.26 à 2.58 ppm (moyenne 1.02) chez les femelles; dans le foie, ces valeurs sont de 0.89 à 18.30 ppm pour les mâles et de 0.55 à 91.30 ppm pour les femelles. Les moyennes pour ces dernières séries sont sans signification. Les auteurs ont de plus mesuré la fraction méthylée sur quelques échantillons (quatre de muscle et six de foie). Dans le muscle, Hg est méthylé presque à 100%; dans le foie, la fraction méthylée varie de 7.4 à 41%, la valeur la plus basse se trouvant dans les foies à plus haute teneur en Hg total.

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HIGH organochlorine pesticide levels were reported in harbour porpoises (*Phocoena phocoena*) from the Bay of Fundy region (Gaskin et al. 1971). Total mercury level analyses of some tissues from these animals are now presented. Although series of samples from other organs will be analyzed at a later date, available results are given as a preliminary information note since there is currently great interest in the biological distribution of this element.

There is now an extensive literature on mercury in the environment, and two comprehensive summaries were recently published, the second dealing specifically with mercury in fish (Löfroth 1970; Berglund et al. 1971). However, papers containing information on mercury in tissues of marine mammals are not numerous (Helminen et al. 1968; Henriksson et al. 1969; Tillander et al. 1970; Bligh and Armstrong 1971).

The harbour porpoise feeds predominantly on clupeoids and gadoids (Rae 1965; Smith 1971), and is an abundant and important component in the upper part of the North Atlantic inshore food web. Porpoises, like seals (Holden 1970), might be used as species to monitor levels of accumulative contaminants in the marine environment. Additionally, porpoise meat is eaten in some eastern fishing communities when the opportunity arises. For both reasons, information is required on the quantitative status of potentially toxic contaminants in porpoises.

Materials and methods — Seventy-three harbour porpoises were collected by D. E. Gaskin in western North Atlantic waters (centered on the Bay of Fundy) in 1969, 1970, and the first half of 1971 as part of an ecological

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and biological study of the species. Muscle samples from 41 and liver samples from 20 of the same animals were chilled to 10 C and frozen to –20 C as soon as field conditions permitted (2–7 hr). A 1-g tissue sample from each specimen was solubilized with a sulphuric–nitric acid mixture (4:1) at 63–65 C. The organomercury bond was oxidized with potassium permanganate in order to eliminate nitric oxides which interfere with mercury determinations. Excess oxidizer was reduced with hydroxylamine and sodium chloride to reduce adsorption of mercury on the glass walls of the apparatus. Metallic mercury was released with stannous chloride. The elemental mercury was driven from the solution by a stream of air and carried through an absorption cell (quartz). Readings of mercury as a cold vapor were made at 253.7 nm using a model AA-5 Techtron Atomic Absorption Spectrophotometer attached to a recorder.

Results — Total Hg levels in muscle tissue of males ranged from 0.21 to 1.92 ppm (average 0.75); in muscle tissue of females from 0.26 to 2.58 (average 1.02); in liver tissue of males from 0.89 to 18.30 ppm; and in liver tissue of females from 0.55 to 91.30 ppm. The range of values and the variances are so large for the liver sample series that averaging would be misleading. In addition, a very few estimations of the methylated fraction of the total mercury were made from four muscle and six liver samples. In the muscle the mercury was virtually 100% methylated (84–100%), but in the liver only 7.4–41% was in this form. The percentage was lowest in those liver samples examined where the concentration of total mercury was highest. While the actual concentration of methyl mercury was greater in specimens with higher total mercury (e.g. in a specimen with 63.90 ppm total Hg, 4.72 ppm was in methylated form), an increasing proportion is present in inorganic form as the total concentration rises.

Total mercury levels in both tissues were roughly proportional to the size of the specimen (Fig. 1, 2), being higher in the larger (and presumably older) animals. The relation between body length in cm and body weight of this population of the species can be expressed as

$$W \text{ lb or } 0.45W \text{ kg} = -93.4 + 1.3L$$

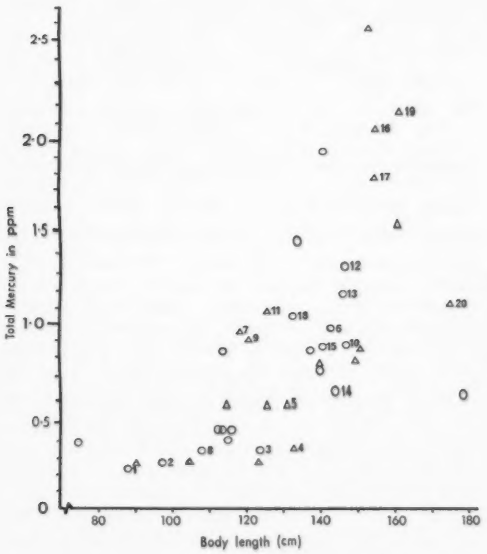

FIG. 1. Total mercury levels in parts per million in longissimus muscle of harbour porpoises *Phocoena phocoena* from the Bay of Fundy region 1969–71, plotted against body length (best analog of age currently available) of specimens. Circles indicate males, triangles females. Numbers beside symbols correspond to those in FIG. 2, and indicate specimens from which both muscle and liver samples were analyzed.

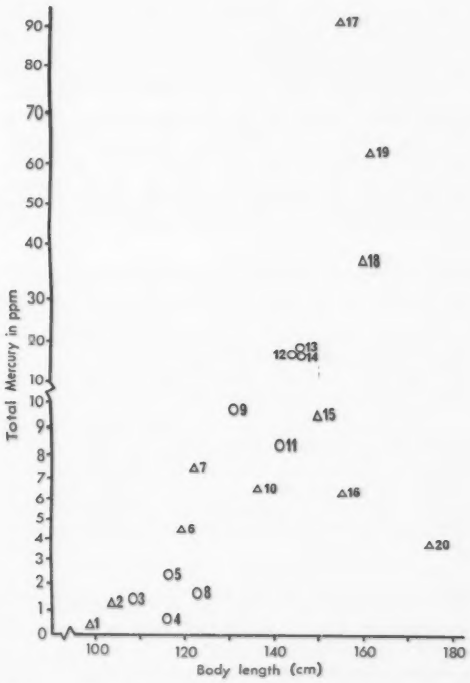

FIG. 2. Total mercury levels in parts per million in livers of harbour porpoises *Phocoena phocoena* from the Bay of Fundy region 1969–71, plotted against body length. Symbols as in FIG. 1.

for males (SE of coefficient ± 0.14, $P < 0.001$)

$$\text{and } W \text{ lb or } 0.45W \text{ kg} = -80.7 + 1.3L$$

for females (SE of coefficient ± 0.01, $P < 0.001$). However, lengths are generally used as it is not possible under prevailing field conditions to weigh every specimen taken. Methods of determining the absolute age of specimens are under study.

Comprehensive data on mercury content of western North Atlantic fish on which harbour porpoises feed are lacking. Animals in different parts of the region could be exposed to quite different levels of contamination; for example, the three specimens taken in 1969 which had the highest liver concentrations (15.30 to 91.30 ppm total Hg) were all caught from the same school. We do not yet know if there are equilibrium levels for individuals, or if there is much variation over a season or shorter period. While the data presented in Fig. 1 and 2 suggest a net gain of mercury with increase in size and/or age, much more study is necessary.

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BERGLUND, F., ET AL. 1971. Methylmercury in fish. A toxicological-epidemiologic evaluation of risks. Report from an expert group. Nord. Hyg. Tidskr. 4: 1–363.

BLIGH, E. G., AND F. A. J. ARMSTRONG. 1971. Marine mercury pollution in Canada. ICES Council Meeting 1971 (E: 34): 1–3. (Mimeo)

GASKIN, D. E., M. HOLDRINET, AND R. FRANK. 1971. Organochlorine pesticide residues in harbour porpoises from the Bay of Fundy. Nature 233: 499–500.

HELMINEN, M., E. KARPPANEN, AND I. KOIVISTO. 1968. Mercury content of the ringed seal of Lake Saima. Suom. Elainlaakarilenti 74: 87–89.

HENRIKSSON, K., E. KARPPANEN, AND M. HELMINEN. 1969. The amounts of mercury in seals from lakes and sea. Nord. Hyg. Tidskr. 50: 54–59. (In Swedish)

HOLDEN, A. V. 1970. Monitoring organochlorine contamination of the marine environment by the analysis of residues in seals. FAO (Food Agr. Organ. U.N.) Tech. Conf. Mar. Pollut. Pap. FIR/MP/70/E:63.

LÖFROTH, G. 1970. Methylmercury. A review of health hazards and side effects associated with the emission of mercury compounds into natural systems. Ecol. Res. Comm. Bull. (Stockholm) 4: 1–38.

RAE, B. B. 1965. The food of the common porpoise (*Phocoena phocoena*). J. Zool. 146: 114–122.

SMITH, G. J. D. MS 1971. The diet and stomach of the harbour porpoise (*Phocoena phocoena* L.). M.Sc. Thesis. Univ. of Guelph, Guelph, Ont. 69 p.

TILLANDER, M., J. K. MIETTINEN, AND I. KOIVISTO. 1970. Excretion rate of methyl mercury in the seal (*Pusa hispida*). FAO (Food Agr. Organ. U.N.) Tech. Conf. Mar. Pollut. Pap. FIR/MP/70/E:67.

Scales as a Means of Aging Dover Sole (*Microstomus pacificus*)

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DEMORY, R. L. 1972. Scales as a means of aging Dover sole (*Microstomus pacificus*). J. Fish. Res. Bd. Canada 29: 1647–1650.

An aging technique by means of scales is described for Dover sole (*Microstomus pacificus*). Agreement after two readings was 50%, but increased to 80% after three readings. Validation of the first annulus was established after examination of juvenile scales over a period of time during the first year of benthic residence. Location of the first annulus is contrary to published accounts. Scales offer a superior method of aging, especially for older fish. Interpretation and accuracy of scales from adult Dover sole males that remain in deep water one or more years is questionable.

DEMORY, R. L. 1972. Scales as a means of aging Dover sole (*Microstomus pacificus*). J. Fish. Res. Bd. Canada 29: 1647–1650.

L'auteur décrit une méthode de détermination de l'âge de la sole de Douvres (*Microstomus pacificus*) à l'aide des écailles. Il y a accord entre deux lectures dans 50% des cas, et dans 80% après trois lectures. L'examen suivi des écailles de jeunes individus durant leur première année permet d'établir la validité du premier anneau de croissance. La position de ce premier anneau ne correspond pas aux descriptions déjà publiées. Les écailles sont supérieures aux autres structures pour la détermination de l'âge, surtout des individus âgés. Cependant, l'interprétation et l'exactitude des écailles de soles mâles qui séjournent un an ou plus en eau salée demeurent problématiques.

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THIS report summarizes results of recent attempts to evaluate the validity of age determination by means of scales of Dover sole (*Microstomus pacificus*), an important species in the Oregon trawl fishery. Since 1957 scales have been used for age determination instead of otoliths because it was thought that scales were better aging structures, especially for older fish. However, analysis of scales from tagged fish at liberty up to 12 years cast doubt on scale interpretation. It appeared that scale interpretation underestimated the age of at least some of the tagged fish.

I collected scales from 374 juvenile and 800 adult sole caught near the mouth of the Columbia River in commercial catches (782 scales were readable). Length ranged from 6 to 60 cm. Scales of juvenile sole of the 1966 year-class were collected in March, July, October, and November, 1966. Scales from adult sole were collected monthly from April to November, 1966. Several dozen scales were removed from the eyed-side of the body of each fish one or two rows dorsal or ventral to the lateral line and midway between the head and tail. Scales from this area are easier to read because they are larger and scale patterns are more clearly defined.

Method — Scales were dry mounted between two glass slides and later examined without further preparation at 39× magnification.

Scales were independently read twice without reference to sex or length, then a third reading was made on those scales in which ages disagreed. Disagreements after three readings were resolved so that each scale had an assigned age.

Two criteria determined an annulus: closely spaced circuli that mark the end of a season's growth and a translucent ring that borders the last of the closely spaced circuli (Fig. 1). Only scales collected from samples of the commercial catch were used to evaluate the aging method.

Usefulness of the method — To validate the first annulus as a true year mark, it was necessary to examine the early life history of Dover sole. Hagerman (1952) and Harry (1959) showed that spawning occurs from November to March with the peak in January. Post larvae are pelagic for several months and juveniles apparently do not become demersal until late fall at a length of 50–55 mm. (Hagerman

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TABLE 1. Comparison of first and second age readings of 782 Dover sole (*Microstomus pacificus*) scales.

Assigned age	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	Total
5	5[a]	1		1																7
6	2	*26*	10	4																42
7	1	12	*54*	16	9	1														93
8	1	2	19	*78*	28	11	2													141
9			5	16	*76*	24	5	2												128
10				7	23	*60*	20	6	3											119
11				1	9	24	*35*	15	5	3										92
12				1	3	3	14	*23*	7	4	2									57
13							3	5	*15*	9	1									33
14								8	9	*8*	3	2								30
15								4	2	2	*6*	1	1							16
16										1	3	*5*	1							10
17											1	1		2		1				5
18														*1*						1
19													1	1		2				4
20														1				1		2
21																			1	1
22																			1	1
23																				
Total	9	41	88	124	148	123	79	63	41	27	16	9	3	5		3		1	2	782

% Agreement: 50.1
% Agreement ± 1: 84.8

[a]No. of agreements italicized.

1952). My earliest catch record of small juveniles within a calendar year was January, 1967, when six fish (average length 66 mm) were caught near the mouth of the Columbia River. The annulus was nearly complete. During the previous November, fish with one annulus averaged 121 mm in length. This validation of the first annulus was contrary to the conclusion of Hagerman (1952), who concluded that the first mark was false and the result of transition from pelagic to demersal life. However, he drew his conclusion from Arora (1951), who demonstrated this for Pacific sanddab (*Citharichthys sordidus*).

Marginal scale growth showed that marks taken as annuli were formed each year as follows:

Month	Number	% With Marginal growth
Apr.	99	6
May	100	9
June	94	39
July	99	88
Aug.	95	86
Sept.	98	96
Oct.	97	100

In November, 32% of the scales (n = 100) showed the new annulus and, in February, 83% of a sample

TABLE 2. Numbers of Dover sole (*M. pacificus*) scales read and percent agreement after three readings.

Assigned age	Males		Females	
	No. read	% agreement	No. read	% agreement
5	2	100.0	4	75.0
6	12	91.6	23	95.7
7	24	100.0	52	88.5
8	33	97.0	86	89.5
9	59	83.1	78	83.3
10	56	91.1	64	78.1
11	44	77.3	49	77.6
12	26	84.6	37	56.8
13	24	66.7	30	56.7
14	9	77.8	20	75.0
15	5	40.0	13	38.5
16	3	33.3	10	40.0
17	1	100.0	5	20.0
18			6	66.7
19			1	0.0
20			1	100.0
21			1	0.0
22			2	50.0
23			1	100.0
24			1	0.0
Total	298	84.6	484	76.7

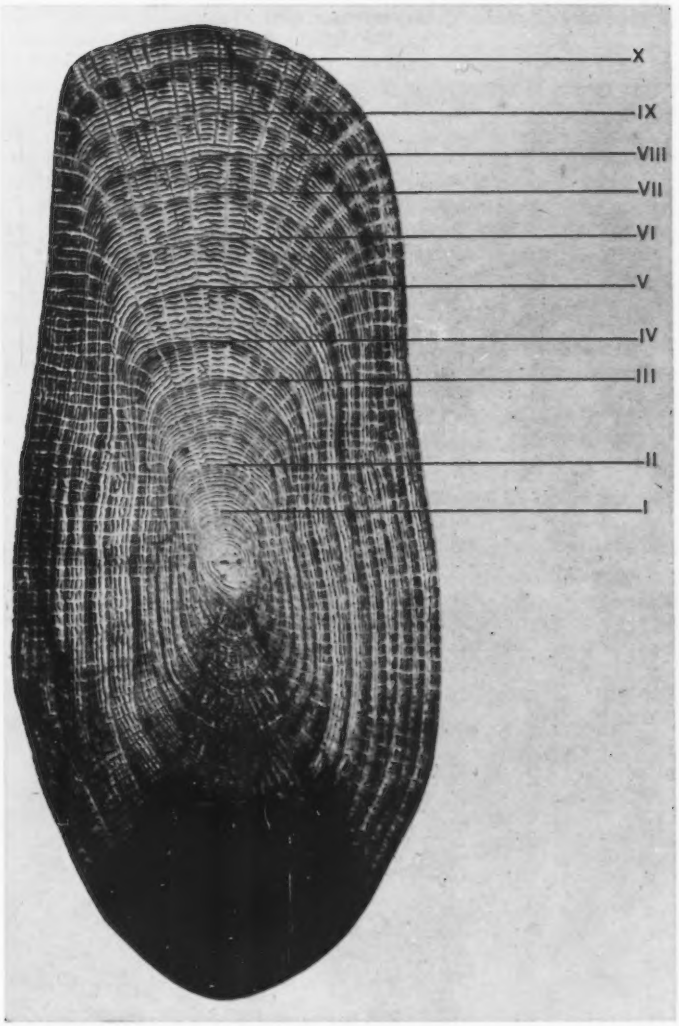

FIG. 1. A scale of a 52-cm female Dover sole (*Microstomus pacificus*) caught in August, 1967. The translucent ring is most apparent on the anterior margin of the 9th annulus.

Demory — J. Fish. Res. Bd. Canada

TABLE 3. Frequency distribution of male and female Dover sole (*M. pacificus*) lengths according to age assigned by scale reading. Fork lengths are adjusted to the nearest lower centimeter.

Length (*cm*)	Age																	Total
	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	>20[a]	
Males																		
31			1															1
32	1	2	3	1				1										8
33		3	4	5		1												13
34		3	5	4	5	5	1											23
35	1		2	3	11	10	2	2			1							32
36		2	6	8	13	6	5	4		1	1							46
37		1	3	4	12	9	5	4	5									43
38				1	10	10	7	2		1			1					32
39		1		4	4	6	7		3	1								26
40				3	3	5	7	2	6	1								27
41					1	1	3	3	1		1	1						11
42						1	1	1	2	1								6
43						1	4	3	3	2								13
44								2	3									5
45						1		2	1	2		1						7
46							1											1
47							1				2	1						4
Total	2	12	24	33	59	56	44	26	24	9	5	3	1					298
Mean length	34.0	34.9	34.9	36.5	37.2	37.8	39.6	39.8	41.0	41.7	41.7	44.8	38.5					38.1
Females																		
27			1															1
28																		
29		1																1
30		1																1
31																		
32	3	1	2	3	1													10
33		4	5	1	1	1												12
34		5	8	12	4	3												32
35	1	6	6	14	10	4	2											43
36		1	5	17	6	6	3	2										40
37		2	11	12	17	4	4	1	1									52
38		1	3	6	6	7	3	5	1									32
39		1	7	9	9	5	4	2	1									38
40			2	5	6	11	2	5	2	2								35
41			1	3	8	10	8	2	3									35
42					6	2	5	2	3	3	1	3						25
43				1	1	6	6	4	2	1	1		1					23
44			1		2	2	5	4	3	1	2	1						21
45				1		3	4	3	5	2	1	1	1					21
46				1			1	3	3		1	2						11
47							1	2	2	2	1				1			9
48				1	1					3	1		1	1				8
49							1	2	2	1				1				7
50									1		2							3
51									1	2	1	1		1				6
52																		
53										2			1				1	4
54											2	1		1				4
55										1		1	1				1	4
56														1			2	3
57																		
58														1		1		2
59																		
60																	1	1
Total	4	23	52	86	78	64	49	37	30	20	13	10	5	6	1	1	5	484
Mean length	33.3	34.9	36.6	37.3	38.6	39.8	41.6	42.6	44.4	47.2	48.0	47.2	49.3	53.2	47.5	58.5		40.4

[a] >20, age/length: 21/56; 22/53,60; 23/56; 24/55.

of small fish (<30 cm, $n = 48$) showed the new annulus. When scales were removed early in the growing season, the new growth sometimes tore away and only fragments remained. Thus, in April and May, the actual percentage of scales showing growth beyond the last annulus might be higher.

Direct evidence of annulus formation was from scales of two tagged fish recaptured 359 and 390 days after tagging. Scales from each fish had been taken at tagging and both fish showed the addition of one annulus at recapture.

For 782 scales read, agreement between the first two readings was 50%, and ± 1 year, 85% (Table 1). Agreement after three readings was 80%. Agreement was 85 and 77% for males and females, respectively (Table 2). For fish through age 10, the average percentage agreement was 94% for males and 85% for females; for older fish, 69 and 49%, respectively. An χ^2 test showed the third reading was not biased by memory.

Age–length relation — Another way used to show the annual addition of year marks was to relate increasing age to increasing length. This relation was positive for both males and females, but poor for males (Table 3). The poor relation between length and age for males can be attributed to either underaging of older fish or a leveling off of the growth.

At times, annuli counts from scales of Dover sole underestimated the true age. Between July, 1962, and July, 1967, 24 Dover sole with scales suitable for aging were recovered from a tagging program conducted in 1955 (Westrheim and Morgan 1963). Fish were at liberty between 7 and 12 years. The estimated age at tagging, based on scales, ranged from 2 to 10 years. In nine cases, the estimated age at tagging, ± 1 year, did not correspond with the length of tagging (Table 4). In all nine cases, scales underestimated the age estimated from lengths. A plausible explanation for the lack of discernible annuli might be related to the migratory behavior of Dover sole. Tag returns from the study by Westrheim and Morgan (1963) and a more recent study by Magill and Milburn (MS 1965) suggest that some fish, especially males,

TABLE 4. Age at recovery and estimated age at tagging (± 1 year) of Dover sole (*M. pacificus*) tagged in 1955.

Recovered (*month/year*)	At liberty (*years, months*)	Length (*cm*)		Sex	Age at recovery	Estimated age at tagging, ± 1 year
		release	recovery			
6/62	7,2	38	38.9	M	12	5
6/62	7,3	38	41.8	M	16	9
10/62	7,6	39	40.0	F	13	6
10/62	7,6	46	49.0	F	17	10
10/62	7,6	39	40.8	M	12	5
1/63	7,10	48	50.8	F	17	10
6/63	8,2	41	44.6	M	14	6[a]
7/63	8,3	43	44.5	M	14	6[a]
8/63	8,4	35	38.0	M	13	5
9/63	8,5	37	38.2	M	12	4[a]
10/63	8,6	38	43.3	M	12	4[a]
2/64	8,10	42	44.3	M	17	9
5/64	9,1	46	45.2	M	18	9[a]
6/64	9,2	41	46.7	F	17	8
10/64	9,6	41	45.4	M	18	9
8/65	9,11	40	42.5	M	17	8
4/65	10,0	37	38.6	M	13	3[a]
6/65	10,2	51	52.7	F	18	8[a]
6/65	10,2	39	42.8	M	17	7
11/65	10,7	33	39.1	M	16	6
11/65	10,7	34	36.6	M	12	2[a]
11/65	10,7	37	39.8	M	14	4[a]
3/66	10,11	38	40.1	M	15	5
7/67	12,2	43	46.3	M	21	9

[a]Estimated age at tagging ± 1 year; does not correspond with the length at tagging. See Table 3 for length range within age group.

remained 1 or more years in deep water, in which case growth was probably minimal.

Conclusions — The level of agreement of scale readings of Dover sole is acceptable in view of work done on other long-lived northeastern Pacific groundfish. Westrheim (MS 1958) obtained 42% agreement after two readings on scales of Pacific ocean perch (*Sebastes alutus*), but this increased to 70% after three readings. Domenowski (MS 1966) obtained 47% agreement in two readings on otoliths of rex sole (*Glyptocephalus zachirus*) and 97% ± 1 year. Palman (1956) had agreement of 93% in two readings with English sole (*Parophrys vetulus*) interopercles and 75% with otoliths.

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ARORA, H. L. 1951. An investigation of the California sanddab *Citharichthys sordidus* (Girard). Calif. Fish Game 37: 3–42.

DOMENOWSKI, R. S. MS 1966. A comparison of age estimation techniques applied to rex sole, *Glyptocephalus zachirus*. M.Sc. Thesis. Univ. Washington, Seattle, Wash. 94 p.

HAGERMAN, F. B. 1952. The biology of the Dover sole, *Microstomus pacificus* (Lockington). Calif. Dep. Fish Game Fish Bull. 85: 48 p.

HARRY, G. Y., Jr. 1959. Time of spawning, length at maturity, and fecundity of the English, petrale, and Dover sole (*Parophrys vetulus*, *Eopsetta jordani*, and *Microstomus pacificus*, respectively). Oreg. Fish Comm. Res. Briefs 7: 5–13.

MAGILL, A. R., AND G. S. MILBURN. MS 1965. Tagging studies to determine the offshore–inshore exchange of groundfish off Oregon and Washington. U.S. At. Energy Comm. Contract AT(4501)1731. Oreg. Fish Comm. Progr. Rep. 4: 22 p. (Mimeogr.)

PALMEN, A. T. 1956. A comparison of otoliths and interopercular bones as age indicators of English sole. Wash. Dep. Fish. Fish. Res. Pap. 1(4): 5–20.

WESTRHEIM, S. J. MS 1958. On the biology of Pacific ocean perch *Sebastodes alutus* (Gilbert). M.Sc. Thesis. Univ. Washington, Seattle, Wash. 106 p.

WESTRHEIM, S. J., AND A. R. MORGAN. 1963. Results from tagging a spawning stock of Dover sole *Microstomus pacificus*. Pac. Mar. Fish. Comm. Bull. 4: 88 p.

Population Biomass, Number of Individuals, Average Individual Weight, and the Linear Surplus-Production Model

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JENSEN, A. L. 1972. Population biomass, number of individuals, average individual weight, and the linear surplus-production model. J. Fish. Res. Bd. Canada 29: 1651–1655.

The identity $B_t = N_t\overline{W}_t$, where B_t is the population biomass, N_t is the population size, and $\overline{W}_t$ is the average individual weight for all ages, is applied to develop simultaneous equations for change in biomass, number of individuals, and average individual weight for the linear surplus-production equation. It is shown that equations for all three variables cannot be simultaneously logistic. The relation between $\log_e N_t$ and $\log_e \overline{W}_t$ predicted by the linear surplus-production model is compared with observations of bluegill population densities and average weights estimated from 10 years of cove rotenone sampling in five large TVA reservoirs. The fit of the model to the data is fairly good, but it accounts for only a small amount of the total variation observed.

JENSEN, A. L. 1972. Population biomass, number of individuals, average individual weight, and the linear surplus-production model. J. Fish. Res. Bd. Canada 29: 1651–1655.

L'auteur utilise la relation $B_t = N_t\overline{W}_t$, dans laquelle B_t représente la biomasse de la population, N_t la grandeur de la population, et $\overline{W}_t$ le poids moyen des individus de tous les âges, pour établir des équations simultanées s'appliquant aux variations de la biomasse, au nombre d'individus et au poids moyen des individus, et en arriver à l'équation linéaire surplus-production. Selon lui, les équations pour ces trois variables ne peuvent être toutes de forme logistique simultanément. L'auteur compare la relation entre $\log_e N_t$ et $\log_e \overline{W}_t$, prédite suivant le modèle surplus-production, avec des données sur la densité des populations et le poids moyen de crapets à oreilles bleues, obtenues à l'aide d'échantillonnages à la roténone dans les anses de cinq grands bassins de retenue de la TVA, sur une période de 10 ans. Le modèle s'ajuste assez bien aux données, mais ne rend compte que d'une faible portion de la variabilité totale observée.

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IN this study the relations among biomass, number of individuals, and average individual weight implied by the linear surplus-production equation for biomass are investigated. The mathematical symbols used are defined below:

- B_t = population biomass,
- B_∞ = environmental carrying capacity in terms of biomass,
- $f(P_t)$ = regulatory function for rate of change in population size,
- F_1 = fishing mortality coefficient for population density,
- F_2 = effect of fishing coefficient for average weight,
- g = instantaneous growth rate,
- i = instantaneous mortality rate,
- k = instantaneous rate of increase in population size, including both change in numbers and change in weight,
- N_t = total number of individuals of all ages,
- N_∞ = environmental carrying capacity in terms of number of individuals,
- P_t = population size in biomass or numbers,
- P_∞ = population size in biomass or numbers at the environmental carrying capacity,
- q = catch coefficient,
- r = instantaneous rate of change in population density,
- $\overline{W}_t$ = average individual weight of all ages,
- $\overline{W}_\infty$ = maximum attainable average individual weight of all ages, and
- X = annual fishing effort.

Surplus-production models describe the dynamics of an exploited population using a function of the population size in which the effects of recruitment, growth, and natural mortality are combined (Graham 1935; Schaefer 1954, 1957; Ricker 1958).

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Schaefer (1954, 1957) developed the following general equation for surplus-production of a fish population exploited under average environmental conditions,

$$\frac{dP}{dt} = P_t f(P_t) - q \times P_t \quad (1)$$

Schaefer (1954) used the linear function,

$$f(P_t) = k\left(\frac{P_\infty - P_t}{P_\infty}\right). \quad (2)$$

Substitution of equation (2) into equation (1) gives the linear surplus-production equation,

$$\frac{dP}{dt} = k P_t \left(\frac{P_\infty - P_t}{P_\infty}\right) - q \times P_t, \quad (3)$$

which is the familiar logistic growth equation with the mortality term $-q \times P_t$ added to account for fishing mortality.

Analysis of equation (3) has led to a comprehensive description of the dynamics of exploited fish populations (Schaefer 1957; Ricker 1958), but Fox (1970) and Pella and Tomlinson (1969) have proposed alternative equations for the regulatory function which may be better than equation (2) in some situations (Silliman 1971).

The general relationship among population biomass, number of individuals, and average individual weight — Population biomass is related to number of individuals and average individual weight by the identity,

$$B_t = N_t \overline{W}_t \quad (4)$$

If B_t, N_t, and $\overline{W}_t$ are differentiable functions of time, applying the rule for differentiating the product of two functions gives a general equation (equation 5) for relating time rate of change in biomass to both change in number of individuals and to change in average individual weight,

$$\frac{dB}{dt} = N_t \frac{d\overline{W}}{dt} + \overline{W}_t \frac{dN}{dt}. \quad (5)$$

Applying equation (5), change in biomass can be separated into its components, which are change in the number of individuals and change in average weight per individual. It is not possible to determine $\overline{W}_t$ and N_t from B_t, nor to determine $N_t \frac{d\overline{W}}{dt}$ and $\overline{W}_t \frac{dN}{dt}$ from $\frac{dB}{dt}$. The approach taken in this study, therefore, is to develop expressions for $N_t \frac{d\overline{W}}{dt}$ and $\overline{W}_t \frac{dN}{dt}$ and then apply equation (5) to determine the appropriate equation for $\frac{dB}{dt}$.

Incidentally, equation (5) provides a simple derivation of Ricker's (1946) equation for rate of change in biomass. If $\frac{d\overline{W}}{dt} = g \overline{W}_t$ and $\frac{dN}{dt} = -i N$, then

$$\frac{dB}{dt} = g N_t \overline{W}_t - i N_t \overline{W}_t = (g - i) B_t, \quad (6)$$

as was shown by Ricker (1946). The equation for change in biomass can similarly be determined for other forms of $\frac{dN}{dt}$ and $\frac{d\overline{W}}{dt}$.

Equation for change in biomass when change in the number of individuals and change in the average weight are both logistic — If the rate of change in the number of individuals and the rate of change in the average weight are both logistic, i.e.,

$$\frac{dN}{dt} = r\left(\frac{N_\infty - N_t}{N_\infty}\right) N_t$$

$$\frac{d\overline{W}}{dt} = g\left(\frac{\overline{W}_\infty - \overline{W}_t}{\overline{W}_\infty}\right) \overline{W}_t$$

then application of equation (5) shows that the rate of change in biomass is given by the equation,

$$\frac{dB}{dt} = B_t\left[r\left(\frac{N_\infty - N_t}{N_\infty}\right) + g\left(\frac{\overline{W}_\infty - \overline{W}_t}{\overline{W}_\infty}\right)\right], \quad (7)$$

which is not a simple logistic equation. Change in the number of individuals in equation (7) is independent of change in the average weight. The carrying capacity, B_∞, of populations where equation (7) applies is determined by the product of N_∞ and $\overline{W}_\infty$. For all initial conditions of N_t and $\overline{W}_t$ the result at equilibrium will be N_∞ individuals with an average weight of $\overline{W}_\infty$. In equation (7) there are really two independent environmental carrying capacities — one for the number of individuals and one for the average individual weight.

The environmental carrying capacity for many fish species appears to be related more closely to biomass standing crop than to the product of a maximum number of individuals and a maximum average weight. Swingle and Smith (1941) showed that, if a large number of young bluegill (*Lepomis macrochirus*) were stocked in farm ponds and harvested after one growth season, the bluegill were small, but, if a smaller number of young bluegill were stocked, the harvested bluegill were large. The total weight of a fish population stocked in ponds apparently adjusts to the food production capacity of the ponds. Nikolskii (1969) concluded that for many commercial fish species in Europe; including sturgeon, herring, salmon, cod, and carp; there is some form of relation between average weight and population density.

Equations for change in number of individuals and average weight when change in biomas is logistic — Substitution in equation (5) shows that if,

$$\frac{dN}{dt} = r N_t \left(\frac{B_\infty - N_t \overline{W}_t}{B_\infty}\right) \quad (8)$$

$$\frac{d\overline{W}}{dt} = g\overline{W}_t\left(\frac{B_\infty - N_t\overline{W}_t}{B_\infty}\right), \quad (9)$$

then change in biomass is given by the logistic equation,

$$\frac{dB}{dt} = (g + r)\,B_t\left(\frac{B_\infty - B_t}{B_\infty}\right). \quad (10)$$

The above development of equation (10) shows that the constant k in Schaefer's model is the sum of the constants for growth in weight, g, and for growth in numbers, r.

Substitution in equation (5) shows that if $\frac{d\overline{W}}{dt} = 0$, then both N_t and B_t can be simultaneously described by logistic equations. This is apparently the situation referred to by Schaefer (1954, 1957) when he states that P_t in equation (1) can be either N_t or B_t. Experience clearly shows, however, that the effects of fishing on a fish population are a change in the age distribution, a decrease in the number of older and larger fish, and a decrease in $\overline{W}_t$ (Baranov 1918; Ricker 1946). The surplus-production model described by equations (8), (9), and (10) is, therefore, more realistic than the model in which N_t and B_t are both logistic and $\frac{d\overline{W}}{dt} = 0$.

Equations (8) and (9) relate average weight and number of individuals by the equation,

$$\frac{dN}{dt} = \frac{rN_t}{g\overline{W}_t} \cdot \frac{d\overline{W}}{dt}, \quad (11)$$

in which the rate of change of $\log_e\overline{W}_t$ is proportional to the rate of change of $\log_e N_t$. Integration of equation (11) gives,

$$\log_e N_t = \frac{r}{g}\log_e\overline{W}_t + (\log_e N_o - \frac{r}{g}\log_e\overline{W}_o). \quad (12)$$

Applying equation (12) to calculate N_t and $\overline{W}_t$ for large values of t and different initial conditions (N_o and $\overline{W}_o$) shows that the environmental carrying capacity, B_∞, can be reached by any combination of $\overline{W}_t$ and N_t whose product is B_∞. Depending on the initial conditions, it is possible to have a large population of small individuals or a small population of large individuals.

The linear regression equation,

$$\log_e N_t = b\log_e\overline{W}_t + c, \quad (13)$$

where $b = \frac{r}{g}$ and $c = \log_e N_o - \frac{r}{g}\log_e\overline{W}_o$, was fitted by least squares to data on bluegill sunfish population density and weight collected by cove rotenone sampling in the fall of the year over a 10-year period in five large TVA reservoirs (Fig. 1). There is considerable scatter about the fitted regression line ($r = -0.37$), but it is clear that; as predicted by equations (8), (9), and (10); large numbers of individuals are associated with a low average individual weight and small numbers of individuals are associated with a high average individual weight. The slope of the regression line is significantly different from zero ($\alpha = 0.05$).

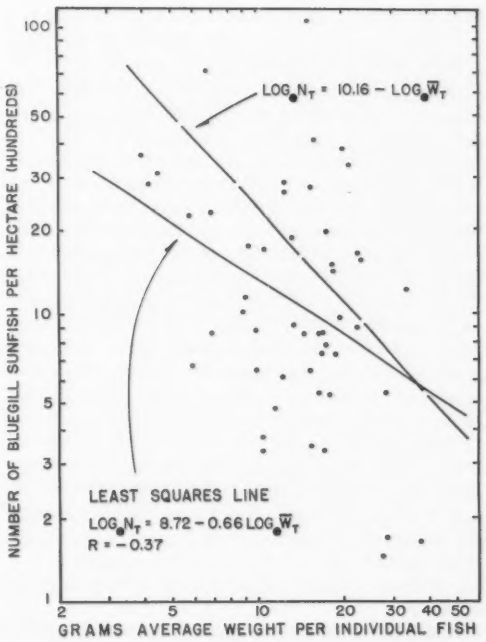

FIG. 1. Relation between number of individuals and average individual weight for bluegill sunfish (*Lepomis macrochirus*) in five large TVA reservoirs. The units of the equation are the actual number of bluegill and not hundreds of bluegill.

A linear relationship between $\log_e N_t$ and $\log_e\overline{W}_t$ can arise in at least one additional way. If an equilibrium exists at the carrying capacity where $B_t = B_\infty$, taking logarithms in equation (4) gives

$$\log_e N_t = \log_e B_\infty - \log_e\overline{W}_t. \quad (14)$$

The logarithm of the carrying capacity, B_∞, was estimated for the bluegill data using the average observed biomass from all observations. The broken line in Fig. 1 is a plot of equation (14) when $\log_e B_\infty = 10.16$. The slope of the least squares equation (equation (13)) does not differ significantly from unity ($\alpha = 0.05$), which indicates that both models (equations (13) and (14)) fit the data equally well.

An unrealistic property of the linear surplus-production model brought out by equation (11) is that the average individual weight has no upper limit. For practical applications to wild fish stocks this property of the model apparently is not too important. But this property may need to be considered if the model is applied to situations where stocking is important.

Discussion—The most important point illustrated in this study is that because $B_t = N_t\overline{W}_t$, sur-

plus-production models for change in population numbers, average weight per individual, and biomass cannot be independently formulated.

There are important differences between the equations developed here and the similar equations for change in individual weight and number of individuals developed in dynamic pool studies. In development of dynamic pool models attention first centers on the population or stock of fish. Recruitment, growth, and mortality are modeled and studied without reference to the environment or the environmental carrying capacity, and these models are useful for understanding many aspects of population processes. But to study the dynamic properties of a fish stock responding to a fishery, water pollutants, or other environmental disturbances, the modeling must be carried a step further to include some form of feedback mechanisms. Most of the fluctuations in fish populations appear to result from variations in recruitment, so fisheries scientists applying dynamic pool models have developed feedback models relating the number of spawners to the number of recruits (Beverton and Holt 1957; Ricker 1958). These feedback models are based on the concept of an environmental carrying capacity.

In the development of surplus-production models the environmental carrying capacity is considered immediately, and the rates of change in biomass, number of individuals, and average individual weight are related directly to this environmental carrying capacity. Processes which occur within the population (e.g. change in age structure or change in individual weight) are not considered.

The equations used to establish the feedback mechanisms in both dynamic pool and surplus-production models are of a similar functional form. The same mathematical equation is often used to describe widely different phenomena. The logistic equation used in the linear surplus-production model is a general model of a simple feedback loop, with both positive and negative feedback, that could be applied to both dynamic pool and surplus-production models, but at different stages in their development.

The surplus-production approach can be applied to study fishing effects on the number of individuals and on the average weight as well as on biomass. The equations for change in the number of individuals and average weight are

$$\frac{dN}{dt} = rN_t - \frac{r\overline{W}_t N_t^2}{B_\infty} - F_1 N_t, \qquad (15)$$

and

$$\frac{d\overline{W}}{dt} = g\overline{W}_t - \frac{g\overline{W}_t^2 N_t}{B_\infty} - F_2\overline{W}_t, \qquad (16)$$

which are simple modifications of equations (8) and (9), where F_1N_t has been introduced to account for the individuals removed by the fishery and $F_2\overline{W}_t$ has been introduced to account for change in the average weight caused by fishing. Substitution of equations (15) and (16) into equation (5) gives Schaefer's equation (equation (3)), where $P_t = B_t$, $k = r + g$, and $qX = F_1 + F_2$.

At equilibrium, where $\frac{dB}{dt} = \frac{dN}{dt} = \frac{d\overline{W}}{dt} = 0$, the biomass yield is obtained as usual from equation (3),

$$Y = qXB_t = kB_t\left(\frac{B_\infty - B_t}{B_\infty}\right), \qquad (17)$$

and the population biomass

$$B_t = B_\infty - \frac{qXB_\infty}{k}. \qquad (18)$$

Applying equations (15) and (16) gives the number of individuals and the average individual weight at equilibrium as

$$N_t = \frac{(r - F_1)B_\infty}{r\overline{W}_t} \qquad (19)$$

and

$$\overline{W}_t = \frac{(q - F_2)B_\infty}{gN_t}. \qquad (20)$$

Generalization of the linear surplus-production model to include the number of individuals and the average individual weight as well as biomass may increase its utility in practical fisheries work, but dynamic pool models still contain more information than even this generalized surplus-production model and dynamic pool models should be the final objective of fisheries studies.

As pointed out by Silliman (1971), surplus-production models are valuable in the early stages of an investigation, but later additional biological information should be obtained to develop a more complete model. Surplus-production models appear to offer the simplest approach for studying the effects on yield of ecological interactions among populations. Larkin (1963), for example, has used this approach to study the effect of interspecific competition on yield.

BARANOV, F. I. 1918. On the question of the biological basis of fisheries. Nauch. issled. ikhtiol. Inst. Izv. 1: 81–128. (In Russian)

BEVERTON, R. J. H., AND S. J. HOLT. 1957. On the dynamics of exploited fish populations. Her Majesty's Stationery Office, London.

FOX, W. W. 1970. An exponential surplus-yield model for optimizing exploited fish populations. Trans. Amer. Fish. Soc. 99: 80–88.

GRAHAM, M. 1935. Modern theory of exploiting a fishery, and application to North Sea trawling. J. Cons. Cons. Perma. Int. Explor. Mer 10: 263–274.

LARKIN, P. A. 1963. Interspecific competition and exploitation. J. Fish. Res. Bd. Canada 20: 647–678.

NIKOLSKII, G. V. 1969. Theory of fish population dynamics. (Transl. from Russian by J. E. S. Bradley, Oliver & Boyd Ltd., Edinburgh, Scotland)

PELLA, J. J., AND P. K. TOMLINSON. 1969. A generalized stock production model. Bull. Inter-Amer. Trop. Tuna Comm. 13: 421–496.

RICKER, W. E. 1946. Production and utilization of fish populations. Ecol. Monogr. 16: 373–391.

1958. Handbook of computations for biological statistics of fish populations. Fish. Res. Board Can. Bull. 119: 300 p.

SCHAEFER, M. B. 1954. Some aspects of the dynamics of populations important to the management of the commercial marine fisheries. Bull. Inter-Amer. Trop. Tuna Comm. 1: 27–56.

1957. A study of the dynamics of the fishery for yellowfin tuna in the eastern tropical Pacific Ocean. Bull. Inter-Amer. Trop. Tuna Comm. 2: 245–285.

SILLIMAN, R. P. 1971. Advantages and limitations of "simple" fishery models in light of laboratory experiments. J. Fish. Res. Bd. Canada 28: 1211–1214.

SWINGLE, H. S., AND E. V. SMITH. 1941. Experiments on the stocking of fish ponds. Trans. N. Amer. Wildlife Natur. Resour. Conf. 5: 267–276.

Unusual Occurrence of the Brook Stickleback (*Culaea inconstans*) in the Mackenzie River, Northwest Territories

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FALK, M. R. 1972. Unusual occurrence of the brook stickleback (*Culaea inconstans*) in the Mackenzie River, Northwest Territories. J. Fish. Res. Bd. Canada 29: 1655–1656.

Two specimens of the brook stickleback (*Culaea inconstans*) were taken in the mouths of Pierre and Tsital Trien creeks near Arctic Red River on the Mackenzie River during 1971. Previous most northerly published records were from the south shore of Great Slave Lake. The specimens may have been carried downstream by spring floods and may not represent a resident population.

FALK, M. R. 1972. Unusual occurrence of the brook stickleback (*Culaea inconstans*) in the Mackenzie River, Northwest Territories. J. Fish. Res. Bd. Canada 29: 1655–1656.

On a capturé en 1971 deux exemplaires de l'épinoche à cinq épines (*Culaea inconstans*) à l'embouchure des ruisseaux Pierre et Tsital Trien, près d'Arctic Red River, sur le Mackenzie. Cette espèce n'avait pas été signalée avant ce jour plus au nord que la rive sud du Grand lac des Esclaves. Ces deux spécimens ont pu avoir été entraînés en aval par les crues de printemps et n'appartiennent pas nécessairement à une population résidante.

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Two specimens of the brook stickleback (*Culaea inconstans*) were taken by a small-mesh beach seine at the mouths of two creeks (67°20′N, 133°22′W; and 67°28′N, 133°35′W) near Arctic Red River on the Mackenzie River in September, 1971. These specimens were collected during a study undertaken by the Department of the Environment, Fisheries Service, to investigate the fish resources of the Mackenzie River Valley.

Total lengths for the specimens were 49 and 59 mm. Fin ray counts for both specimens fell within the range reported by Nelson (1969). These were: dorsal V, 9; anal I, 9; pelvic I, 1; and pectoral, 10. Dorsal and pelvic spines were very short, as is

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characteristic of northern populations (Nelson 1969). Both were females with developing eggs and both had empty stomachs.

These specimens extend the known range of the species (McPhail and Lindsey 1970) by approximately 460 miles north, or 1000 miles down the Mackenzie River from the south shore of Great Slave Lake. It is doubtful that the range extends past the Mackenzie delta since the species has a low salinity tolerance (Nelson 1968). The only route by which the species could have reached the Arctic Red River area is down the Mackenzie River. It is possible that the specimens were carried downstream by spring floods. Despite intensive seining along the Mackenzie and in associated tributaries, especially around Norman Wells and Fort Simpson, no other specimens were collected (Hatfield et al. 1972). The sparse occurrence of two individuals at different locations suggests that the species may not be resident.

The specimens were collected in turbid backwater areas at the mouths of Pierre and Tsital Trien Creeks, entering the east side of the Mackenzie River at a water temperature of 5 C. Both locations had a sand–silt bottom and were void of aquatic vegetation. According to Winn (1960), brook sticklebacks form large aggregations and are invariably associated with dense vegetation in clear slow streams and shallow lakes. Similar areas were located in the headwater areas of these creeks, but *C. inconstans* was absent from seine catches and was not observed in these areas. The specimens were caught in locations where ninespine sticklebacks (*Pungitius pungitius*) were present in large numbers around tributary mouths along the Mackenzie River (Hatfield et al. 1972).

The specimens were deposited in the National Museum of Natural Sciences, Ottawa, Canada.

Acknowledgments — The author appreciates assistance from Mr R. J. Paterson in criticizing the manuscript and Dr C. C. Lindsey, Department of Zoology, University of Manitoba, in identifying the specimens.

HATFIELD, C. T., J. N. STEIN, M. R. FALK, AND C. S. JESSOP. 1972. Fish resources of the Mackenzie River Valley, Interim Report 1.Vol . 1. Department of the Environment, Fisheries Service, Winnipeg, Man. 247 p.

MCPHAIL, J. D., AND C. C. LINDSEY. 1970. Freshwater fishes of northwestern Canada and Alaska. Fish. Res. Board Can. Bull. 173: 381 p.

NELSON, J. S. 1968. Salinity tolerance of brook sticklebacks, *Culaea inconstans*, freshwater ninespine sticklebacks, *Pungitius pungitius*, and freshwater fourspine sticklebacks, *Apeltes quadracus*. Can. J. Zool. 46: 663–667.

1969. Geographic variation in the brook stickleback, *Culaea inconstans*, and notes on nomenclature and distribution. J. Fish. Res. Bd. Canada 26: 2431–2447.

WINN, H. E. 1960. Biology of the brook stickleback, *Eucalia inconstans* (Kirtland). Amer. Midland Natur. 63: 424–438.

Paralytic Shellfish Poison and Melanin Distribution in Fractions of Toxic Butter Clam (*Saxidomus giganteus*) Siphon[1]

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PRICE, R. J., AND J. S. LEE. 1972. Paralytic shellfish poison and melanin distribution in fractions of toxic butter clam (*Saxidomus giganteus*) siphon. J. Fish. Res. Bd. Canada 29: 1657–1658.

Frozen toxic butter clam (*Saxidomus giganteus*) siphons were fractionated and each fraction was bioassayed for paralytic shellfish poison (PSP) and chemically analyzed for melanin. Sonication removed over 50% of the melanin from the siphons and this fraction contained nearly 50% of the PSP initially present in the siphons. The data presented further implicate melanin as a PSP binding agent in vivo.

PRICE, R. J., AND J. S. LEE. 1972. Paralytic shellfish poison and melanin distribution in fractions of toxic butter clam (*Saxidomus giganteus*) siphon. J. Fish. Res. Bd. Canada 29: 1657–1658.

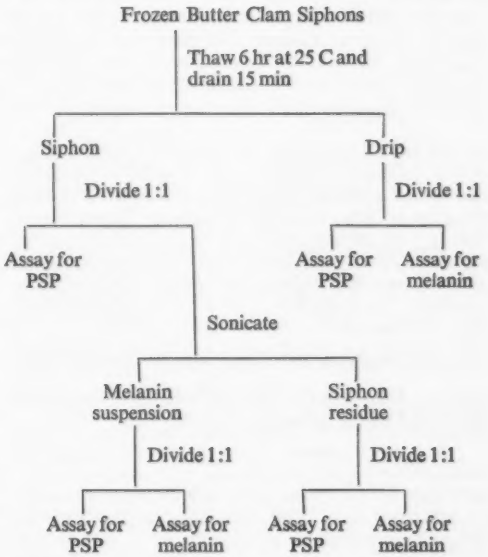
Nous avons fractionné des siphons de coques de Washington (*Saxidomus giganteus*) toxiques congelés et mesuré sur chaque fraction le toxique paralysant des mollusques (PSP) par essai biologique, et la mélanine par analyse chimique. Le traitement par les ultrasons enlève plus de 50% de la mélanine, et cette fraction contient près de 50% du PSP initial des siphons. Nos résultats fournissent une preuve de plus que la mélanine est l'agent fixateur du PSP in vivo.

Received February 14, 1972

PARALYTIC shellfish poison (PSP) is not evenly distributed in the toxic butter clam and as much as 88% of the PSP will accumulate in the siphon of the clam. In a toxic siphon, PSP is concentrated in the siphon tip and the concentration decreases in proportion to the distance from the tip (Quayle 1969). In butter clams (*Saxidomus giganteus*), the areas of PSP concentration appear to correspond to the areas of melanin pigmentation indicated by Comfort (1951).

Since our evidence suggested that the site of PSP binding in butter clams was melanin granules (Price and Lee 1971), the present investigation was undertaken to determine the distribution of PSP in fractions of toxic butter clam siphons in relation to that of melanin, and thus to determine if PSP is associated with melanin in vivo.

Materials and Methods — Frozen toxic butter clam siphons (2233 g) were obtained from Alaska and contained approximately 10,000 mouse units PSP/100 g. The siphons were thawed and fractionated as follows:



Melanin was removed from the siphons by sonication (Price and Lee 1971). Each of the fractions was mixed

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with an equal volume of distilled water, homogenized, adjusted to *p*H 3.0 with 5-N HCl and frozen at −20 C.

PSP concentrations in each fraction were determined by the standard mouse bioassay (Horwitz 1960).

Melanin from the corresponding fractions was purified by papain treatment (Price and Lee 1971). The purified melanin granules were washed twice by centrifugation with distilled water, and dehydrated by washing the granules once with 50% aqueous ethanol, twice with 95% ethanol, and twice with acetone. The dehydrated melanin was dried under N_2 at room temperature.

The colorimetric assay for melanin was a modification of that of Ruban et al. (1969). Dried melanin was dissolved in 0.5-N NaOH by incubating the mixture at 50 C for 2 hr in a shaking water bath. After cooling to room temperature, melanin was precipitated from the solution by adjusting the *p*H to 2.0 with 5-N HCl. The precipitate was washed twice by centrifugation with 0.01-N HCl, and dissolved in 90 ml of 0.5 N-NaOH at 50 C. After 1-hr incubation, the volume was adjusted to 100 ml with 0.5-N NaOH, the solution was filtered through a sintered glass filter, and the melanin concentration was determined by measuring the absorbance of the solution at 420 nm with a spectrophotometer.

Results and discussion — The distribution of PSP and melanin in the fractions obtained from frozen toxic butter clam siphons is shown in Fig. 1. Sonication has been shown to be an effective means of removing melanin from the butter clam siphon (Price and Lee 1971), and the sonication filtrate which contained 53% of the siphon melanin also contained 46% of the PSP in the siphons. The parallel distribution of PSP and melanin in the siphon fractions suggests that they may be closely associated in vivo in toxic butter clam siphons.

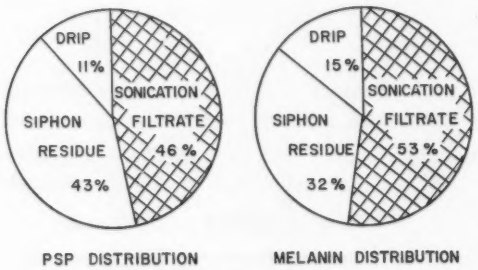

FIG. 1. Distribution of PSP and melanin in toxic butter clam (*Saxidomus giganteus*) siphon.

Twarog et al. (1972) reported that species of molluscs which have nervous systems with tetrodotoxin(TTX)-resistant spike mechanisms have all been implicated in outbreaks of paralytic shellfish poisoning in people, and that insensitivity to TTX appears to be associated with a resistance to PSP. Several species which are known to become toxic with PSP, however, were sensitive to TTX and PSP. The authors suggested that in some sensitive species the PSP may be stored in such a way as to reduce circulating levels of toxin and thus protect their nervous systems from exposure. If PSP interacts with the melanin granules in butter clam siphons in vivo in a manner similar to that described for the interaction of PSP and melanin in vitro (Price and Lee 1971; Price and Lee 1972), then the melanin in the butter clam siphon may function as a protective mechanism for the clam.

Acknowledgments — This investigation was supported in part by the National Oceanic and Atmospheric Administration (Administered by the Department of Commerce), Institutional Sea Grant GH 2-35187, under the graduate research assistantship program.

COMFORT, E. H. 1951. The pigmentation of molluscan shells. Biol. Rev. (Cambridge) 26: 285–301.

HORWITZ, W. [ed.]. 1960. Official methods of analysis of the Association of Official Agricultural Chemists. 9th ed. P.O. Box 540, Benjamin Franklin Station, Washington, D.C. 832 p.

PRICE, R. J., AND J. S. LEE. 1971. Interaction between paralytic shellfish poison and melanin obtained from butter clam (*Saxidomus giganteus*) and synthetic melanin. J. Fish. Res. Bd. Canada 28: 1789–1792.

1972. Effect of cations on the interaction between paralytic shellfish poison and butter clam (*Saxidomus giganteus*) melanin. J. Fish. Res. Bd. Canada 29: 1659–1661.

QUAYLE, D. B. 1969. Paralytic shellfish poisoning in British Columbia. Fish. Res. Board Can. Bull. 168: 68 p.

RUBAN, E. L., S. P. LYAKH, I. M. KHRULEVA, AND I. A. TITOVA. 1969. Melanin pigments of *Nadsoniella nigra*. (Abstr.) Izv. Akad. Nauk SSSR 1: 134–148.

TWAROG, B. M., T. HIDAKA, AND H. YAMAGUCHI. 1972. Resistance to tetrodotoxin and saxitoxin in nerves of bivalve molluscs. Toxicon 10: 273–278.

Effect of Cations on the Interaction Between Paralytic Shellfish Poison and Butter Clam (*Saxidomus giganteus*) Melanin[1]

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PRICE, R. J., AND J. S. LEE. 1972. Effect of cations on the interaction between paralytic shellfish poison and butter clam (*Saxidomus giganteus*) melanin. J. Fish. Res. Bd. Canada 29: 1659–1661.

The interaction between paralytic shellfish poison (PSP) and butter clam (*Saxidomus giganteus*) melanin was strongly influenced by the presence of cations. The amount of PSP bound by the melanin decreased by 68% in the presence of Al^{+++}, 62 and 57%, respectively, with Ca^{++} and Ba^{++}, and 17 and 15% with Na^{+} and K^{+}. Salt solutions containing 0.01-M Al^{+++}, Mg^{++}, Ca^{++}, Ba^{++}, Na^{+}, or K^{+} induced the desorption of PSP bound to melanin by 54, 34, 28, 19, 11, and 4%, respectively. These results confirm our earlier conclusion that the PSP–melanin interaction is reversible and electrostatic in nature.

PRICE, R. J., AND J. S. LEE. 1972. Effect of cations on the interaction between paralytic shellfish poison and butter clam (*Saxidomus giganteus*) melanin. J. Fish. Res. Bd. Canada 29: 1659–1661.

La présence de cations affecte grandement l'interaction du toxique paralysant des mollusques (PSP) avec la mélanine de la coque de Washington (*Saxidomus giganteus*). La quantité de PSP fixé par la mélanine diminue de 68% en présence d'Al^{+++}, 62 et 57%, respectivement, en présence de Ca^{++} et Ba^{++}, et 17 et 15% avec Na^{+} et K^{+}. Des solutions de sels contenant 0.01-M Al^{+++}, Mg^{++}, Ca^{++}, Ba^{++}, Na^{+}, ou K^{+} provoquent la désorption du PSP fixé par la mélanine dans des proportions de 54, 34, 28, 19, 11, et 4%, respectivement. Ces résultats sont en accord avec notre conclusion déjà mise de l'avant que l'interaction PSP–mélanine est réversible et électrostatique.

Received February 14, 1972

AN interaction between PSP and natural and synthetic melanin has been previously reported (Price and Lee 1971). The interaction was electrostatic in nature and was strongly influenced by *p*H. Since the interaction between PSP and melanin may play an important role in the retention of PSP by butter clams (*Saxidomus giganteus*), the present investigation was undertaken to further characterize the nature of the PSP–melanin interaction.

The binding of PSP by melanin in the presence of cations was studied to determine how these cations would affect the PSP–melanin interaction, and thus to more clearly define the electrostatic nature of the interaction. The effect of cations on a PSP–melanin complex was investigated to determine the possibility of desorbing bound PSP with salt solutions.

Materials and methods — A purified suspension of butter clam melanin was prepared according to the method of Price and Lee (1971). Melanin granules were removed from the epithelial cells of split butter clam siphons by sonication. The melanin suspension was washed by centrifugation at 20,000 *g* with a $\frac{1}{10}$ dilution of McIlvaines standard buffer at *p*H 6.0 (Lange 1967), treated with Papain (E. C. 3.4.4.10, Sigma Chemical Co.) to remove protein, washed by centrifugation with 0.1-N HCl until the supernatant gave a negative ninhydrin reaction, and suspended in 0.1-N HCl to contain 1.5 mg melanin by dry weight/ml.

Purified powdered PSP was dissolved in 0.001-N HCl (70 μg/ml), and stored at 4 C. A solution containing 100 μg/ml PSP was used as a standard.

PSP solutions were assayed with the Folin-Ciocalteau phenol reagent (Fisher Chemical Co.) as described previously (Price and Lee 1971). Concentrations of PSP from 1 to 15 μg/ml could be measured accurately (Price MS 1972).

Butter clam melanin suspensions in 0.1-N HCl were washed by centrifugation at 20,000 *g* for 20 min three

[1]Technical Paper 3263, Oregon Agricultural Experiment Station. Based on a thesis submitted by the senior author in partial fulfillment of the requirements for the degree of Doctor of Philosophy from the Department of Food Science and Technology, Oregon State University, Corvallis, Oreg. 97331, USA.

Printed in Canada (J2444)

times with buffer prior to binding experiments. The buffers used throughout the PSP–melanin studies were a $\frac{1}{10}$ dilution of McIlvaines standard buffer at *p*H 6.0 (Lange 1967), prepared from 0.01-M citric acid and 0.02-M disodium phosphate. In experiments showing the influence of salts on binding, buffered 0.01-M nitrate solutions were used both for washing melanin and for binding and desorption experiments.

The binding of PSP to melanin and the release of bound PSP from melanin were measured by the method of Price and Lee (1971). Melanin–PSP complexes were washed once with 3-ml portions of buffer at *p*H 6.0 prior to desorption experiments. Controls containing melanin alone and PSP alone were included in all experiments.

Results and discussion — The effect of cations on PSP–melanin binding is shown in Table 1. Cations interfered with the binding of PSP to butter clam melanin and the degree of interference was directly related to the valence of the cation. Salt solutions caused desorption of PSP–melanin complexes and desorption of PSP was directly related to the cation valence. In both series of experiments, the order of decreasing cation activity was trivalent, divalent, and monovalent.

These results confirm our earlier conclusion (Price and Lee 1971) that the PSP–melanin interaction is reversible and electrostatic in nature. The data indicate the reversible nature of the binding, since as much as 54% of the bound PSP could be desorbed by washing the PSP–melanin complex once with 3 ml of 0.01-M aluminum nitrate. The weak nature of the binding is shown by the slight desorption that takes place when the PSP–melanin complex is washed with the same buffer used in the adsorption of PSP onto the melanin.

TABLE 1. Effect of cations on the interaction between PSP and clam melanin.

Cation (0.01 M)	μg PSP bound[a]	% Decrease in binding	μg PSP desorbed[b]	% Net desorption
Control	19.4	0	1.5	0
K^+	16.5	15	2.0	4
Na^{++}	16.2	17	2.8	11
Ba^{++}	8.4	57	3.6	19
Ca^{++}	7.3	62	4.9	28
Mg^{++}	–[c]	–	5.1	34
Al^{+++}	6.2	68	8.2	54

[a]PSP (27 μg) and clam melanin (4.5 mg), *p*H 6.0.
[b]4.5 mg of clam melanin contained 12 μg of bound PSP. Melanin–PSP complex was washed with 3-ml portions of 0.01-M nitrate solutions in citrate–phosphate buffer at 25 C.
[c]Not included in this experiment.

Schantz suggested that the binding of PSP to butter clams was similar to the binding of PSP on a cation exchange resin (E. J. Schantz personal communication). We reported that interactions between PSP and natural and synthetic melanin closely resembled that noted between PSP and live butter clams and between PSP and cation exchange resins: maximum adsorption was observed above *p*H 6.0, and there was minimum adsorption at *p*H 3.0 (Price and Lee 1971). Rapid desorption of bound PSP occurred at *p*H 3.0, and the rate of desorption decreased as the *p*H increased. The results shown in Table 1 support a cation exchange reaction mechanism, and are consistent with the proposed weak cation exchanging nature of melanin (White 1958). The affinity of a cation for the cation exchanger increases with increasing valence, and this effect is greater in the case of weak acid cation exchangers than with strong acid ones (Bruenger and Atherton 1967). Mono-, di-, and trivalent cations interfere with PSP–melanin binding and cause desorption of bound PSP from melanin (Table 1). These effects increase with increasing valence, suggesting a competition for the binding sites on melanin. The increased competition observed with increased valence is consistent with the preference of weak acid cation exchangers for cations of higher valence (Bruenger and Atherton 1967).

Further support for the cation exchange nature of the PSP–melanin interaction can be found in the order of cation interference. There is an apparent relationship between the ability of cations to compete with PSP for binding sites on melanin, and the hydrated ionic radii of the cations. Although the differences in the amounts of PSP bound or desorbed in the presence of cations of the same valence are small, and may not be significant, there is an apparent increase in competition with decreased hydrated ionic radius. This characteristic is typical of cation exchange reactions (Bruenger and Atherton 1967).

The results of these experiments together with the effects of *p*H on the interaction between PSP and melanin, reported previously (Price and Lee 1971), suggest a possible reason for the ability of butter clams to bind and retain PSP for extended periods. PSP may be bound by melanin in butter clams in a manner similar to that described for the interaction of PSP and melanin in vitro. An electrostatic interaction of this nature would explain not only the retention of PSP by butter clams, but also the ability of acid to remove PSP from toxic clams (Schantz et al. 1957), since the interaction is greatly influenced by *p*H (Price and Lee 1971). The weak nature of the PSP–melanin interaction would account for the reported gradual reduction in toxicity

of butter clams in the absence of toxic dinoflagellates (Quayle 1969).

The reversibility of the PSP–melanin interaction in the presence of cations or hydrogen ions suggests a means for detoxification of toxic clams. Although soaking of minced or whole toxic clams in seawater at a *p*H as low as 5.0 has been shown to be ineffective in reducing PSP levels in clam meats (U.S. Dep. Interior 1966), no attempts have been made to detoxify butter clams with salt solutions of polyvalent cations. Since PSP interacts electrostatically with melanin in vitro and may bind similarly in vivo, it may be possible to reduce the PSP levels in toxic butter clams by incubating live clams or soaking minced clams in solutions containing polyvalent cations which would compete with the PSP for binding sites in the clam. A process of this type may be more practical than one involving low *p*H (U.S. Dep. Interior 1966) or removal of butter clam siphons and extended heat processing (Dassow 1966) to reduce PSP levels.

Acknowledgments — We are greatly indebted to Dr E. J. Schantz, University of Wisconsin, Madison, for the supply of purified PSP. This investigation was supported in part by the National Oceanic and Atmospheric Administration (administered by the Department of Commerce) Institutional Sea Grant GH 2-35187 under the graduate research assistantship program.

BRUENGER, F. W., AND D. R. ATHERTON. 1967. The incorporation of various metals into *in vivo*- and *in vitro*-produced melanin. Radiat. Res. 32: 1–12.

DASSOW, J. A. 1966. Processing detoxification, p. 12. *In* W. A. Felsing, Jr. [ed.] Proceedings of Joint Sanitation Seminar on North Pacific Clams. Alaska Dep. H.E.W., U.S. Dep. H.E.W., P.H.S.

LANGE, N. A. 1967. Handbook of chemistry. McGraw-Hill, New York, N.Y. 2001 p.

PRICE, P. J. MS 1972. Interaction between paralytic shellfish poison and clam melanin and protein. Ph.D. Thesis. Oregon State Univ. Corvallis, Oreg. 82 p.

PRICE, R. J., AND J. S. LEE. 1971. The interaction between paralytic shellfish poison and melanin obtained from butter clam (*Saxidomus giganteus*) and synthetic melanin. J. Fish. Res. Bd. Canada 28: 1789–1792.

QUAYLE, D. B. 1969. Paralytic shellfish poisoning in British Columbia. Fish. Res. Board Can. Bull. 168: 68 p.

SCHANTZ, E. J., J. D. MOLD, D. W. STANGER, J. SHAVEL, F. J. RIEL, J. P. BOWDEN, J. M. LYNCH, R. S. WYLER, B. RIEGEL, AND H. SOMMER. 1957. Paralytic shellfish poison. VI. A procedure for the isolation and purification of the poison from toxic clam and mussel tissues. J. Amer. Chem. Soc. 79: 5230–5235.

U.S. DEPARTMENT OF THE INTERIOR. Fish and Wildlife Service. Bureau of Commercial Fisheries. 1966. Acid pre-treatment to remove paralytic shellfish poison from butter clams (*Saxidomus giganteus*). Tech. Rep. 72: 4 p.

WHITE, L. P. 1958. Melanin: a naturally occurring cation exchange material. Nature (London) 182: 1427–1428.

Recent Articles on FRB Investigations Published in Other Periodicals

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating FRB establishment. The number following each title refers to the FRB establishment listed below.

(1) Fisheries Research Board of Canada
Biological Station
P.O. Drawer 100
Nanaimo, British Columbia

(2) Fisheries Research Board of Canada
Vancouver Laboratory
6640 N.W. Marine Drive
Vancouver 8, British Columbia

(3) Fisheries Research Board of Canada
Pacific Environment Institute
4160 Marine Drive
West Vancouver, British Columbia

(4) Fisheries Research Board of Canada
Freshwater Institute
501 University Crescent
Winnipeg 19, Manitoba

(5) Fisheries Research Board of Canada
Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue, Quebec

(6) Fisheries Research Board of Canada
Biological Station
St. Andrews, New Brunswick

(7) Fisheries Research Board of Canada
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Bedford Institute
P.O. Box 1006
Dartmouth, Nova Scotia

(8) Fisheries Research Board of Canada
Halifax Laboratory
P.O. Box 429
Halifax, Nova Scotia

(9) Fisheries Research Board of Canada
Biological Station
St. John's, Newfoundland

1707. DUEDALL, I. W. 1972. The partial molal volume of calcium carbonate in sea water. Geochim. Cosmochim. Acta 36: 729–734. (7)

1708. MANN, K. H. 1972. Ecological energetics of the sea-weed zone in a marine bay on the Atlantic coast of Canada. II. Productivity of the seaweeds. Mar. Biol. 14: 199–209. (7)

1709. MANN, K. H. 1972. Case history: the River Thames, p. 215–232. *In* River ecology and man. Academic Press, Inc., New York, N.Y. (7)

1710. WILDISH, D. J. 1972. Acute toxicity of polyoxyethylene esters and polyoxyethylene ethers to *S. salar* and *G. oceanicus*. Water Res. 6: 759–762. (6)

1711. SYMONS, P. E. K. 1971. Behavioural adjustment of population density to available food by juvenile atlantic salmon. J. Anim. Ecol. 40: 569–587. (6)

1712. KATES, M., A. J. HANCOCK, R. G. ACKMAN, AND S. N. HOOPER. 1972. Preparation and characterization of the DDD(RRR)-stereoisomer of 4,8,12,16-tetramethylheptadecanoic acid and 5,9,13,17-tetramethyloctadecanoic acid. Chem. Phys. Lipids 8: 32–41. (8)

1713. GORDON, L. I., P. K. PARK, S. W. HAGER, AND T. R. PARSONS. 1971. Carbon dioxide partial pressures in North Pacific surface waters–time variations. J. Oceanogr. Soc. Jap. 27: 81–90. (1)

1714. ACKMAN, R. G., S. N. HOOPER, AND J. HINGLEY. 1972. Isomer subfractionation effects in the study of longer chain monoethylene fatty acids in hydrogenated oils. J. Chromatogr. Sci. 10: 430–436. (8)

Additional FRB Publications

The Fisheries Research Board of Canada also publishes a series of *Bulletins*, each on a specific topic. These are for sale by Information Canada, Ottawa, and remittances must be in advance, payable in Canadian funds to the order of the Receiver General of Canada. *Bulletins* are also available from local booksellers and Information Canada bookshops throughout Canada.

The *Annual Report* of the Board and the biennial *Review* of investigations are available free, on request. All publications may be referred to at Board establishments located at: Ottawa; Nanaimo, Vancouver, and West Vancouver, B.C.; Winnipeg, Man.; Ste. Anne de Bellevue, Que.; St. Andrews, N.B.; Halifax and Dartmouth, N.S.; and St. John's, Nfld.

L'Office des recherches sur les pêcheries du Canada publie également une série de *Bulletins*, chacun traitant sur un sujet spécifique. Les publications de l'ORP sont vendues par Information Canada à Ottawa. Les remises, payables d'avance en monnaie canadienne, doivent être établies à l'ordre du Receveur général du Canada. Les *Bulletins* sont aussi en vente chez votre libraire et dans les librairies d'Information Canada.

Vous pouvez obtenir le Rapport Annuel et la Revue biennale gratuitement. On peut consulter ces ouvrages aux établissements de l'Office situés a Ottawa (Ont.) à Nanaïmo, à Vancouver, et à Vancouver Ouest (C.-B.), à Winnipeg (Man.), à Ste-Anne-de-Bellevue (P.Q.), à St-Andrews (N.-B.), à Halifax et à Dartmouth (N.-E.), et à St-Jean (T.-N.).

Journal
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Research
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Canada

Guide to Authors

Details on typing and other items in preparing copy are given in a guide for typists. Also, a list of recommended spellings of about 100 words common in fisheries journals is available from the Office of the Editor.

Editorial Practices

Abstracts should be provided in both English and French.

The *Style Manual for Biological Journals* (2nd ed. American Institute of Biological Sciences, 2000 P St. NW., Washington, D.C.) is, with few exceptions, used as the authority in matters of form. *Webster's Third New International Dictionary* is the standard for spelling and division of words in English; *le Grand Larousse Encyclopédique*, for those in French. Exceptions are made for manuscripts that follow the *Oxford English Dictionary* entirely. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. Manuscript reports are included in the list of references, but are identified both there and in the text by the letters "MS" before the year. The *Journal* follows the spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970), Special Publication No. 6 of the American Fisheries Society.

Notes are distinguished from *papers* on the basis of scope or completeness, rather than quality, of the research. They may be on problems with narrow, readily defined limits; on fishing gear or methods, or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued before completion; or on preliminary findings that warrant publication before problems are resolved satisfactorily. Or they may be used to establish priority in publication. They may be organized with as much flexibility as papers except that they may have no centered headings. They should take no more than four pages in the *Journal*.

In general, *series titles* should be avoided.

Illustrations

Line drawings are preferably made with black india ink on white drawing or tracing paper or on coordinate paper with blue grids. All lines, symbols, letters, and numerals must be neat, and legible when the illustration is reduced to the appropriate size. Drawings should be grouped, when feasible, for economy of space. Originals must be submitted and should be less than 20 inches in the greater dimension for full-page illustrations, and proportionately smaller for those taking less than a page. Drawings must not be rolled for mailing.

Prints of photographs should be made on glossy paper with a wide range in tone. They should be cropped, squared, and mounted with rubber cement on white cardboard, with no space between those arranged in groups.

Proofs and Reprints

Galley proof is sent to the author, and he is responsible for detecting errors in printing as well as in content.

Fifty reprints, without covers, are supplied free. Additional copies needed must be ordered, on the form provided, when the galley proof is returned.

Checklist on Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors. The same questions are recommended to referees who review manuscripts for the *Journal*.

General

Is there new knowledge in the report worth publishing?

Are the findings, interpretations, and conclusions sound, and relevant to the purpose of the study?

If the manuscript is merely confirmatory, is it worth publishing?

Does the manuscript need to be rewritten before the work can be assessed?

Is the contribution placed in the proper perspective concerning the state of knowledge of the subject?

Is the manuscript too long?

Are all the tables and figures needed, and organized for economy of space?

Would any of the text be clearer if condensed?

Should some of the data be made available separately, in a manuscript report or at a data repository?

Should the manuscript be condensed as a Note?

Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics?

Are there inconsistencies between tables or figures and the text, or within the text?

If the manuscript is from a thesis, does it retain characteristics common in these but not desirable in journals?

Parts of the Manuscript

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Has the *introduction* all the important guideposts for orienting the reader?

Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data obtained?

Are generalizations on the *results* adequate?

Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

If there are loopholes in interpretation, are they acknowledged? Should they be overcome?

Should other interpretations be recognized?

Journal of the Fisheries Research Board of Canada

Guide des auteurs

Des détails sur le tapage et certains autres item sont donnés dans le "Guide pour les secrétaires d'auteurs." Aussi, une liste d'épellations recommandées d'environ 100 mots relatifs aux articles sur les pêcheries est disponible au bureau du Directeur de la publication.

Disposition générale

Les résumés doivent être fournis en anglais et en français.

Le *Style Manual for Biological Journals* (deuxième édition, publié par l'American Institute of Biological Sciences, 2000, rue *P* N.-O., Washington, D.C.), a été adopté comme guide des auteurs. Le *Third New International Dictionary* de Webster, dans le cas de l'anglais, et le *Grand Larousse Encyclopédique*, dans le cas du français, font respectivement autorité pour l'orthographe et la séparation des mots. Une exception est faite à l'égard des travaux en anglais qui se conforment entièrement à l'*Oxford English Dictionary*. Les titres de périodiques sont abrégés selon la *Biological Abstracts*. Toute référence à la littérature doit suivre le système nom-année. Les rapports manuscrits sont inclus dans la liste des références, les lettres "MS" avant l'année les identifient dans les références et dans le texte. Le *Journal* se sert des épellations recommandées dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No 6 de l'American Fisheries Society.

Les *notes* se distinguent des écrits d'après l'étendue de la recherche plutôt que par la qualité de la recherche. Les notes peuvent être sur des travaux de recherche de portée restreinte, susceptibles de découler d'investigations plus considérables, pour la description de techniques de recherche et de méthodes de pêche, pour l'établissement de priorités de publication, et pour faire connaître les renseignements utiles tirés d'entreprises qui ont été interrompues avant d'avoir été achevées. Si la portée de la Note le permet, elle peut être disposée comme un mémoire. D'autre part, la disposition est flexible. La longueur ne doit pas dépasser quatre pages imprimées.

En général, les mémoires en série doivent être évités.

Les illustrations

Les dessins au trait doivent être exécutés à l'encre de chine sur papier à dessin blanc ou sur papier à calquer, ou sur papier à coordonnées avec quadrillage bleu. Les lignes, les symboles, les lettres et les chiffres doivent tous être propres. Ils doivent être lisibles quand ils sont réduits à la grandeur voulue pour la reproduction désirée. Les dessins doivent être groupés, si la chose est possible, de façon à utiliser tout l'espace disponible. Les originaux doivent être soumis et devraient mesurer moins de 20 pouces dans le sens le plus long pour les illustrations de pages entières, et proportionnellement plus petit pour les illustrations de moins d'une page. Les dessins doivent être postés à plat.

Les épreuves des photographies doivent être tirées sur papier glacé et accuser une gamme étendue de teintes. Elles doivent être coupées, dressées et montées, au moyen de dissolution, sur carton blanc, sans laisser d'espace entre celles qui sont disposées par groupes.

Epreuves et tires a part

Les épreuves en placards sont adressées à l'auteur et la responsabilité de toute erreur qui peut y rester lui incombe.

Cinquante tirés à part, sans couverture, sont fournis gratuitement. L'auteur peut profiter du renvoi des épreuves en placards pour commander d'autres tirés à part.

Liste de contrôles pour manuscrits

Une attention particulière portée sur les questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les rédacteurs. Les mêmes questions sont recommandées aux arbitres qui font la critique des livres pour le *Journal*.

Generalites

Est-ce que les connaissances nouvelles du rapport méritent d'être publiées?

Les découvertes, les interprétations et les conclusions sont-elles solides et pertinentes au but de l'étude?

Si le manuscrit est tout simplement corroboratif, mérite-t-il d'être publié?

Le manuscrit doit-il être récrit avant d'évaluer le travail?

La contribution est-elle placée dans une perspective appropriée relative à l'état des connaissances du sujet?

Le manuscrit est-il trop long?

Les tables et figures sont-elles toutes nécessaires et arrangées pour économiser le plus d'espace?

Le texte serait-il plus clair s'il était condensé?

Est-ce qu'une partie des données devrait être disponible séparément, soit dans un rapport ou dans un dépôt des données?

Le manuscrit devrait-il être condensé en une note?

Est-ce que la préparation du manuscrit suit la logique mentionnée dans l'introduction?

Y a-t-il des exposés sommaires au début de chaque section et paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents aux sujets?

Y a-t-il des contradictions entre les tableaux et les figures du texte, ou dans le texte même?

Si le manuscrit est tiré d'une thèse, contient-il des caractéristiques communes à cette dernière, mais indésirables dans un journal?

PARTIES DU MANUSCRIT

Le titre renferme-t-il le contenu du rapport?

Le résumé donne-t-il l'essentiel des connaissances nouvelles?

L'introduction a-t-elle tous les points importants afin d'orienter le lecteur?

Le renvoi bibliographique se limite-t-il à la définition du problème?

Les détails du matériel et des méthodes employées sont-ils limités à un minimum pour que les scientifiques comprennent le but de l'étude et puissent porter un jugement adéquat sur les données obtenues.

Les généralisations sur les résultats sont-elles adéquates?

Les conclusions sont-elles déduites des résultats?

La discussion est-elle limitée à l'interprétation et la signification de ces découvertes?

S'il y a des échappatoires dans l'interprétation, sont-elles reconnues? Devraient-elles être maîtrisées?

Devrait-on accepter d'autres interprétations?

Journal Fisheries Research Board Canada

Guide for Typists Preparing Copy

Read the "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 8.5 × 11 inches (21.5 × 28 cm). If possible, use paper with the lines numbered in the margin. Leave all margins at least 1 inch (2.5 cm) wide. Use a nylon or carbon ribbon and keep the typewriter keys clean.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numbers. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (Appendix A). Type the abstract on a separate page (Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send two copies (the ribbon copy and a carbon copy or a photocopy) to the Office of the Editor.

Tables

Examine the current volume of the *Journal* to see how tables are set up (Appendix C).

Note especially the following concerning *numbered tables*:

Type each table on a separate page, using paper without the lines numbered.

Number the tables with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, they are typed with the text, they seldom need horizontal lines, and the data are arranged in as few rows as possible (Appendix C).

Illustrations

Send the original and two clear copies of each illustration with the manuscript. At the lower left of each figure and copy, give the author's name and the figure number. Also type each caption with its figure for identification. For photographs, type this information on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas

Leave three spaces above and below lines that have much material in superscripts or subscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter l and the numeral one, and between the capital letter O and the numeral zero. Make other explanatory notes similarly.

References

Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style that is used in the *Journal* (Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list, but if an abbreviation is in doubt, type the name in full.

Spellings

A list of about 100 words common in fisheries journals is given in Appendix F.

Appendices

Appendices A to F are available on request from the Office of the Editor.

Journal of the Fisheries Research Board of Canada

Guide pour les secrétaires d'auteurs

Lisez le "Guide des auteurs" dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de taper ou d'arranger les détails, suivez la pratique du *Journal*.

Tapez le manuscrit sur un papier blanc et non un papier pelure, mesurant de préférence 8½ × 11 po. (21.5 × 28 cm) et laissant une marge d'au moins 1 po. (2.5 cm). Si possible, employez un papier avec des lignes numérotées dans la marge. Employez un ruban nylon ou carbone et gardez les clés du dactylo nettes.

Chaque partie du manuscrit doit être tapée à double-interligne au moins. La chose est particulièrement importante dans le cas des tableaux, légendes d'illustrations, renvois au bas de la page et listes de références bibliographiques. Evitez de souligner à moins que la matière ne soit en italique. Mettez en retrait la première ligne de chaque paragraphe du texte, ainsi que de chaque légende et renvoi.

Dans les titres et en-têtes, n'employez la majuscule que pour le premier mot, les noms propres et les adjectifs. Pour les petites majuscules, utilisez les petites lettres.

Indiquez tous les renvois au texte par des chiffres arabes en-tête. Numérotez-les consécutivement à travers le texte en commençant par ceux de la page-titre. Placez chaque renvoi immédiatement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit donner que le titre du sujet, le nom et l'affiliation de l'auteur ou des auteurs, et tout autre renvoi utile (Appendice A). Tapez le résumé sur une page séparée (Appendice B). Numérotez chaque page, débutant avec la page-titre.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et un double du travail au Directeur de la publication.

Tableaux

Consultez une copie du *Journal* pour savoir composer les tableaux et placer les lignes qui y entrent (Appendice C).

Portez une attention particulière à ce qui suit concernant les *tableaux numérotés*.

Tapez les tableaux sur des pages séparées, en utilisant du papier sans lignes numérotées.

Numérotez les tableaux avec des chiffres arabes.

Employez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, et très rarement ailleurs. N'employez jamais de lignes verticales; de préférence, laissez de l'espace supplémentaire.

Indiquez les renvois dans les tableaux au moyen de petites lettres italiques en position supérieure et tapez-les au bas de la page.

Placez les tableaux après la liste de références.

A l'endroit où un tableau est mentionné pour la première fois, tapez dans la marge "Tableau (- - -) près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, ils sont tapés avec le texte. Ils ont rarement besoin de lignes horizontales, et l'information est préparée de façons à prendre le moins de rangées possibles.

Illustrations

Joignez au manuscrit expédié le dessin original des illustrations et des photocopies en deux exemplaires.

Au bas et à gauche de chaque figure et photocopie, inscrivez le nom de l'auteur et le numéro de la figure. Aussi, tapez chaque légende avec sa figure pour fin d'identification. Pour les photographies, tapez l'information sur une lisière de papier et collez-la au dos de la photographie. N'écrivez pas et ne tapez pas sur la photographie même. Tapez ensemble sur une feuille séparée et par ordre consécutif les légendes des illustrations, et intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, tapez dans la marge "Fig. (- - -) près d'ici."

Equations, formules et expressions mathematiques

Laissez trois espaces au-dessus et au-dessous des lignes qui contiennent du matériel en positions supérieures ou en positions inférieures, et au-dessus et au-dessous d'équations et de formules. Dans les différentes expressions, placez chaque composante dans sa position appropriée (Appendice D).

Indiquez les symboles clairement. Dans la marge, épelez les lettres grecques la première fois qu'elles apparaissent et faites une distinction entre la lettre "l" et le chiffre "un" et entre la majuscule "O" et le

chiffre "zéro." Procédez de la même façon pour d'autres notes explicatives.

References

Vérifiez le texte afin d'assurer que sont mentionnées toutes les références ou renvois de la liste. Tapez, à doubles interlignes et de la façon prévue (Appendice E) pour le *Journal*, la liste de références. Les noms de journaux sont abrégés suivant la *Biological Abstracts*, mais s'il y a doute, écrivez le nom au long.

Vocabulaire

Une liste d'environ cent mots relatifs aux articles sur les pêcheries est disponible au bureau du Directeur de la publication (Appendice F).

Appendices

Les appendices de A à F sont disponibles sur demande au bureau du Directeur de la publication.

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Antibiotic toxicity, efficacy, and teratogenicity in adult spring chinook salmon (*Oncorhynchus tshawytscha*) **M. G. DeCew** 1513–1517

Methylmercury in northern pike (*Esox lucius*): distribution, elimination, and some biochemical characteristics of contaminated fish **W. L. Lockhart, J. F. Uthe, A. R. Kenney, and P. M. Mehrle** 1519–1523

Respiratory adaptations of prenatal young in the ovary of two species of viviparous seaperch, *Rhacochilus vacca* and *Embiotoca lateralis* **P. W. Webb and J. R. Brett** 1525–1542

Oxygen consumption of embryos and parents, and oxygen transfer characteristics within the ovary of two species of viviparous seaperch, *Rhacochilus vacca* and *Embiotoca lateralis* **P. W. Webb and J. R. Brett** 1543–1553

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Distribution and morphological variation of *Lampsilis radiata* (Pelecypoda, Unionidae) in some central Canadian lakes: a multivariate statistical approach **R. H. Green** 1565–1570

Developmental stages of *Caligus clemensi* (Copepoda: Caligidae) **Z. Kabata** 1571–1593

Some effects of artificial fertilization on enclosed plankton populations in Marion Lake, British Columbia **M. Dickman and I. E. Efford** 1595–1604

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Food specialization by individual trout **J. E. Bryan and P. A. Larkin** 1615–1624

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